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HENNEGUYA VITIENSIS N.SP., A MYXOSPORIDIAN FROM A FIJIAN MARINE FISH, *LEIOGNATHUS FASCIATUS* (LACÉPÈDE, 1803)[1]

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INTRODUCTION

During an entomological and parasitological survey in Fiji in May and June 1949, a visit was made to the airport of Nandi on the western coast of the island of Viti Levu. On the evening of 14th June, I purchased an assortment of small fish from some Indians seining on the sandy flats of Nandi Bay. Smears of the heart blood of these fish were examined for haematozoa on my return to New Zealand. A myxosporidian was then found to be present in the preparations from 12 of 40 specimens of *Leiognathus fasciatus* (Lacépède, 1803). The specimens from which the smears were taken ranged in length from 95 to 140 mm, 150 mm being given by Weber and Beaufort (1931) as the maximum length for the species. There was no apparent correlation between the presence or degree of infection and the size of the host.

Apart from the presence of the myxosporidians, described below as *Henneguya vitiensis* n.sp., there are no artifacts suggesting external contamination in my preparations. It is thus assumed that the parasites are most probably derived from cysts located in some part of the heart. I have been able to trace only three previous records of myxosporidians being found in this organ. Leydig (1851: in Kudo, 1920) discovered a myxosporidian of uncertain genus and species in the auriculoventricular valve of *Leuciscus rutilus* Linnaeus (roach) in Europe. Keysselitz (1908) described *Myxobolus cordis* from the muscle of the ventricle and also, but rarely, that of the bulbus arteriosus, of *Barbus fluviatilis* Agassiz (barbel) in Germany. The third record, that of Ganapati (1938, 1941), is the only one concerning a member of the genus *Henneguya*. This author described *Henneguya otolithi* from the bulbus arteriosus of two Indian marine fishes, *Otolithus ruber* (Bloch, Schneider) and *O. maculatus* (Cuvier and Valenciennes). Both Keysselitz and Ganapati found a scattered infiltration of spores in the kidney, the former author also finding the same condition in the liver and spleen.

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In contrast to their rarity in the heart, MYXOSPORIDIA are commonly found in tissues near this organ, notably in the gills and subcutaneous connective tissue and muscles. Thus, pending an examination of fresh material of *Leiognathus fasciatus* for cysts of *Henneguya vitiensis* n.sp., the possibility of these being located in some tissue in the vicinity of the heart cannot be altogether disregarded. Unfortunately, my preparations were made under very poor lighting conditions which would have rendered the detection of myxosporidian cysts difficult, even if these were being specially searched for.

METHODS

All preparations were made in the following manner. First of all the heart was exposed from the ventral surface by means of a scissors dissection. Its outer wall was wiped dry with a cloth to prevent the contamination of the smear with pericardial fluid, before an incision was made. In all cases the heart was still pulsating when exposed. Following the incision the end of a clean slide was lightly touched against the blood as it welled out, care being taken to prevent the slide from touching any part of the body of the fish. The smears were air-dried, fixed in absolute methyl alcohol and stained with Giemsa.

Henneguya vitiensis n. sp.

(Plate 1, Figures 1–17)

Pansporoblasts: Uninucleate stages (fig. 1) range from 2.0 to 3.1 μ in diameter. The nuclei in these and older pansporoblasts are karyosomatic. With Giemsa the karyosome appears as a small central or eccentric body staining deep red, while there are several intensely staining chromatin masses on the nuclear membrane. Binucleate forms (fig. 2) having a large and a small nucleus and measuring some 3.3 by 2.7 μ, and tetranucleate ones (fig. 3) with two large and two small nuclei and ranging from 5.0 to 6.5 μ in diameter, closely resemble the parallel stages described for *Henneguya wisconsinensis* (host, American yellow perch, *Perca flavescens*) by Mavor and Strasser (1916). Octonucleate pansporoblasts (figs. 4, 5) range in size from 8.0 to 9.5 μ by 6.5 to 7.6 μ, and may contain two densely staining somatic residual nuclei in addition to the eight generative nuclei (fig. 5).

Spore formation: Pansporoblasts in which spore formation is commencing (fig. 6) measure some 14 by 11 μ. These have twelve karyosomatic generative nuclei and four somatic residual nuclei which are not, however, always apparent. The cytoplasm now becomes aggregated into two masses of irregular outline (figs. 7, 8), each of these coming to contain six karyosomatic nuclei and having one or two small extranuclear chromatin masses which probably represent the disintegrating somatic residual nuclei (fig. 8).

Each of the developing spores assumes a more or less rounded shape, and two adjacent vacuoles are formed in the cytoplasm towards one end of the body (fig. 9). These vacuoles represent the developing polar capsules, and each has a polar capsule nucleus closely associated with it. The isthmus between the vacuoles, and the cytoplasm in their immediate vicinity, assumes a deeper blue color with Giemsa than the remainder of the body. In the earliest forms having the shell developed (right hand example, fig. 10) the sporoplasm with its two nuclei is clearly differentiated, in the posterior half of the spore cavity.

The characteristic posterior prolongations of the shell valves first appear as rather blunt, thick projections, the young spore at the same time assuming a pyriform shape (left hand example, fig. 10). Such young spores bear a general resemblance to the corresponding stages of other species of *Henneguya (sensu lato)* as described and figured by previous authors. Ward's (1919) figure of the young spore of *Henneguya salminicola,* Parisi's (1912) figure of that of *(Henneguya)* = *Myxobilatus gasterostei* and Herrick's (1941) account of that of (Henneguya) = *Myxobilatus rupestris* are notable in this connection. The shell valve nuclei usually lie close together at the narrow posterior end of the spore body (right hand example, fig. 11; left hand example, fig. 12), although they may be widely separated from one another (left hand example, fig. 11; right hand example, fig. 12). Frequently only one of these nuclei is apparent (left hand example, fig. 10), perhaps because it directly overlies the other.

In most examples studied the two spores developing from each pansporoblast are at markedly different stages of development (figs. 10, 11). The tail processes become elongated, those

of each spore almost invariably being of the same length (fig. 11). Favorably stained material shows that a very narrow posterior extension of the spore cavity projects into each tail process (figs. 11, 12, etc.). The anterior vacuoles lose their initial round or ovoid shape and become pyriform, and the developing polar filaments may be seen within them (left hand example, fig. 11; fig. 12). These developing filaments have the same staining reaction as the germinative nuclei. They appear as elongated structures situated along the long axes of the polar capsules, and have up to three masses of densely staining chromatic material separated by lighter areas. During the early stages of the formation of the filaments the polar capsule nuclei become closely adherent to the capsular wall (fig. 12) and often appear to be intimately associated with the developing filaments (right hand example, fig. 12). The pyriform polar capsules are initially convergent (left hand example, fig. 11; fig. 12).

Spores: Fully developed spores have smaller bodies and longer tail processes than younger forms. The spore wall is thick, especially so posteriorly, and does not show longitudinal striations. The sutural wall is never thrown into folds. While the outer wall of the spore body is pyriform in outline the cavity itself is more or less ovoid, its narrow posterior extensions into the tail processes usually appearing as thin blue staining lines and often being only partially distinguishable (right hand example, fig. 16). The spore of *Henneguya nüsslini* Schuberg and Schröder, 1905, described from *Trutta fario* Linnaeus (trout) in Germany, is stated by the authors to have a "dark part" running into the tail extensions from the spore cavity. This is probably of similar nature to the posterior extensions of the cavity in the species under discussion. These extensions are seen as distinct tubes, one of which dips behind the other into the ventrally placed tail process, in the lightly stained spore seen in fig. 17.

The tail processes themselves are very slender, and are usually straight or only slightly curved. In most cases they are adherent, one being placed directly behind the other so as to give the impression of there being a single extension only as in the genus *Unicauda* Davis, 1944 (right hand example, fig. 13; fig. 16). The tail process in *Unicauda* is apparently composed of material of separate origin to the shell valves, and has a completely different staining reaction to these with Giemsa (Ward, 1919; Kudo, 1934; Davis, 1944). In *Henneguya vitiensis* the shell valves and their tail processes stain a uniform clear blue with Giemsa. This is the normal staining reaction for the genus, as restricted by Davis (1944) to include only those species having the ends of the shell valves prolonged into more or less extended processes, and the spore body compressed parallel to the sutural plane. The spore of *Henneguya zschokkei* (Gurley, 1894), from European species of *Coregonus* (whitefish), is described as having a tail process either bifurcated along its entire length or single, no intermediate forms occurring (Kudo, 1920). It is possible that what is here regarded as a single tail process is really composed of two such processes closely adherent throughout their lengths, the usual condition in *H. vitiensis*. Spores of the latter species frequently have the tail processes separated at their posterior extremities for a very short distance only (fig. 14). Meglitsch (1937), in his description of *Henneguya limatula* from *Ictalurus* spp. (channel catfish) in Illinois, stated that the long slender tail process "shows a bifurcation in a few cases." From a comparison of this author's fig. 16 with the example of *H. vitiensis* illustrated in fig. 14 of the present paper, it seems possible that the apparently single or terminally bifurcated tail processes of *H. limatula* are composed of two elements closely adherent throughout all or most of their lengths. Occasionally a part of the ventrally placed process of *H. vitiensis* appears to one side of the dorsally placed one (left hand example, fig. 13). Only rarely are the two processes separated throughout their lengths (fig. 15), and when they are it is probably because they have been displaced during smearing.

In fully developed spores (figs. 13–17) the initially convergent polar capsules often appear to be parallel or even slightly divergent. These capsules usually assume an intense purplish black color with Giemsa, the filaments themselves being completely obscured (figs. 13, 16). Occasional lightly stained examples show the polar capsule nuclei, which like those of *H. otolithi* persist for a considerable time (Ganapati, 1941), as large round bodies staining a uniform deep blackish red (figs. 14, 15). Sometimes the filaments themselves may be made out (figs. 15, 17), three or four spiral turns being distinguishable.

The sporoplasm occupies the posterior third to half of the spore cavity. Its anterior margin follows the outline of the posterior ends of the polar capsules, a narrow central projection sometimes extending anteriorly between these capsules (fig. 13). A vacuole of variable size and shape is present in the sporoplasm, the nuclei of which are seldom apparent. Autogamy has taken place in the spores seen in fig. 15, the sporoplasm of each of these having a single nucleus or synkaryon adjacent to the vacuole.

Cohn (1895) figured two spores of *H. oviperda* (from *Esox lucius* Linnaeus, pike, in Europe) in association with one another, their tail processes apparently being terminally adherent (figure reproduced by Kudo, 1920). Ganapati (1941) figured a similar association (his text fig. 3,

22) for *H. otolithi.* He stated that spores carried away by the blood after the rupturing of cysts located near the bulbus arteriosus, and found in scattered infiltrations in the connective tissue of the kidney, are generally associated in pairs. Ganapati suggested that such association may be explained by the completion of the development of scattered pansporoblasts in new surroundings. In all material examined from the 12 parasitized specimens of *Leiognathus fasciatus,* the two spores derived from each pansporoblast always remain in association, even when fully developed and after autogamy has taken place (fig. 15). The spore bodies adhere near the edges, and sometimes the tail processes of the two spores are terminally contiguous (fig. 13).

It is unfortunate that no fresh material of *H. vitiensis* is available for measurement, for a certain amount of shrinkage is an inevitable accompaniment of the methods of fixation and staining employed (Kudo, 1921). The dimensions of the spore, from the measurement of 50 fixed and stained examples, are as follows.—

spore body;	length,	13.7 μ (12.1–15.8 μ)
	breadth,	7.8 μ (6.7– 8.8 μ)
tail processes;	length,	29.1 μ (22.7–35.5 μ)
spore cavity;	length,	8.3 μ (7.1– 9.6 μ)
	breadth,	5.8 μ (5.0– 6.9 μ)
polar capsules;	length,	3.2 μ (2.7– 3.7 μ)
	breadth,	1.7 μ (1.5– 2.0 μ)

DISCUSSION

Kudo (1933) defined the genus *Henneguya* Thélohan, 1892, as follows: "Spore ovoidal, flattened. With a single or double caudal prolongations. Two pyriform polar capsules at anterior end. Sporoplasm with an iodinophilous vacuole. Typically histozoic parasites in fresh-water fish." Davis (1944) subdivided the genus, restricting the name *Henneguya,* as has already been mentioned, to those species having the ends of the shell valves prolonged into more or less extended processes, and the spore body compressed parallel to the sutural plane. This author established the genus *Unicauda* for those species having a single tail process which is not an extension of the shell valves and is apparently composed of different material from these; and the genus *Myxobilatus* for those species having the ends of the shell valves prolonged into tail like processes which are separate throughout their entire length, and the plane of flattening at right angles to the sutural plane. The genera *Unicauda* and *Myxobilatus* have ten and seven species respectively, while *Henneguya* has about 60, including some incompletely described forms and some bare generic records. None of the species of *Unicauda* or *Myxobilatus* are known from the heart, and all are parasites of fresh water fishes.

Members of the genus *Henneguya* (*sensu stricto*) have rarely been collected from marine fish. Apart from *H. otolithi,* the only species previously described from the heart, there are only two records of the genus from fish of a purely marine habit. Parisi (1912) described *H. neapolitana* from *Box salpa* (Cuvier and Valenciennes) (sea bream) at Naples, and Jameson (1929) recorded *Henneguya* sp? from *Sebastodes melanopus* (Girard) (rock cod) in Monterey Bay, California. With regard to fish spending part of their lives in the sea, both Atlantic and Pacific salmon are known as hosts for *Henneguya* in North America. Ward (1919) described *H. salminicola* from *Oncorhynchus kisutch* Walbaum (silver salmon) in Alaska; this myxosporidian now being known from several species of *Oncorhynchus* from collecting stations ranging from the Columbia River to southeastern Alaska (Fish, 1939). Fantham et al. (1939) described *H. salmonis* from *Salmo salar* (Atlantic salmon) in Quebec.

The spores of *H. vitiensis* bear a rather close resemblance to those of a number

of other species of *Henneguya* in their general morphology and dimensions. Those of *H. salvelini,* described from *Salmo salvelinus* Linnaeus (charr) in Lake Constance, Switzerland, by Zandt (1923), of *H. zschokkei* (Gurley, 1894), and of *H. salmonis* all bear a superficial resemblance to the spores of *H. vitiensis,* from which, however, they differ in having very much longer polar capsules. From the descriptions given by Ward (1919) and Fish (1939), the spores of *H. salminicola* bear close comparison with those of the species under discussion. In both species the shell is unstriated and thick, but sutural folds, which do not occur in *H. vitiensis,* were present in 70% of the spores of *H. salminicola* studied by Fish. This author stated that the sporoplasm extends well up between the polar capsules in *H. salminicola,* which is sometimes the case in *H. vitiensis* as well. The two long tail processes of the former species were stated by Ward (1919) to be equal in length, but by Fish (1939) to be markedly unequal. The latter is never the case in the parasite of *Leiognathus fasciatus.* A further point of difference between the two species is that in *H. salminicola* the polar capsules of individual spores are consistently unequal in length, whereas in *H. vitiensis,* although the two capsules are frequently of unequal size, this is by no means always the case. The average dimensions of the polar capsules in the latter species are smaller than those of *H. salminicola.* In fixed and stained preparations those of *H. vitiensis* measure 3.2 by 1.7 μ, as compared with 3.71 by 2.29 μ and 4.35 by 2.29 μ for *H. salminicola.* Indeed, the capsules of the former species are very much smaller than those of almost all other members of the genus. They are comparable with those of *H. brevis* Thélohan, 1892 (1.4 to 5.0 μ in length), a parasite of *Gasterosteus* spp. (sticklebacks) in France, and (judging from Kudo's fig. 524) those of *H. miyairii* Kudo, 1920, of *Carassius auratus* (goldfish) in Japan. The species in which the dimensions of the polar capsules most closely resemble those of *H. vitiensis* is Ganapati's *H. otolithi* (3–4 μ by 2–2.5 μ in fresh material, and thus presumably smaller in fixed and stained preparations). Although the general dimensions of the spores of these two species are again very similar to one another, *H. otolithi* is quite distinct from *H. vitiensis* in having a characteristic transverse thickening at about the middle of the spore body (Ganapati, 1941).

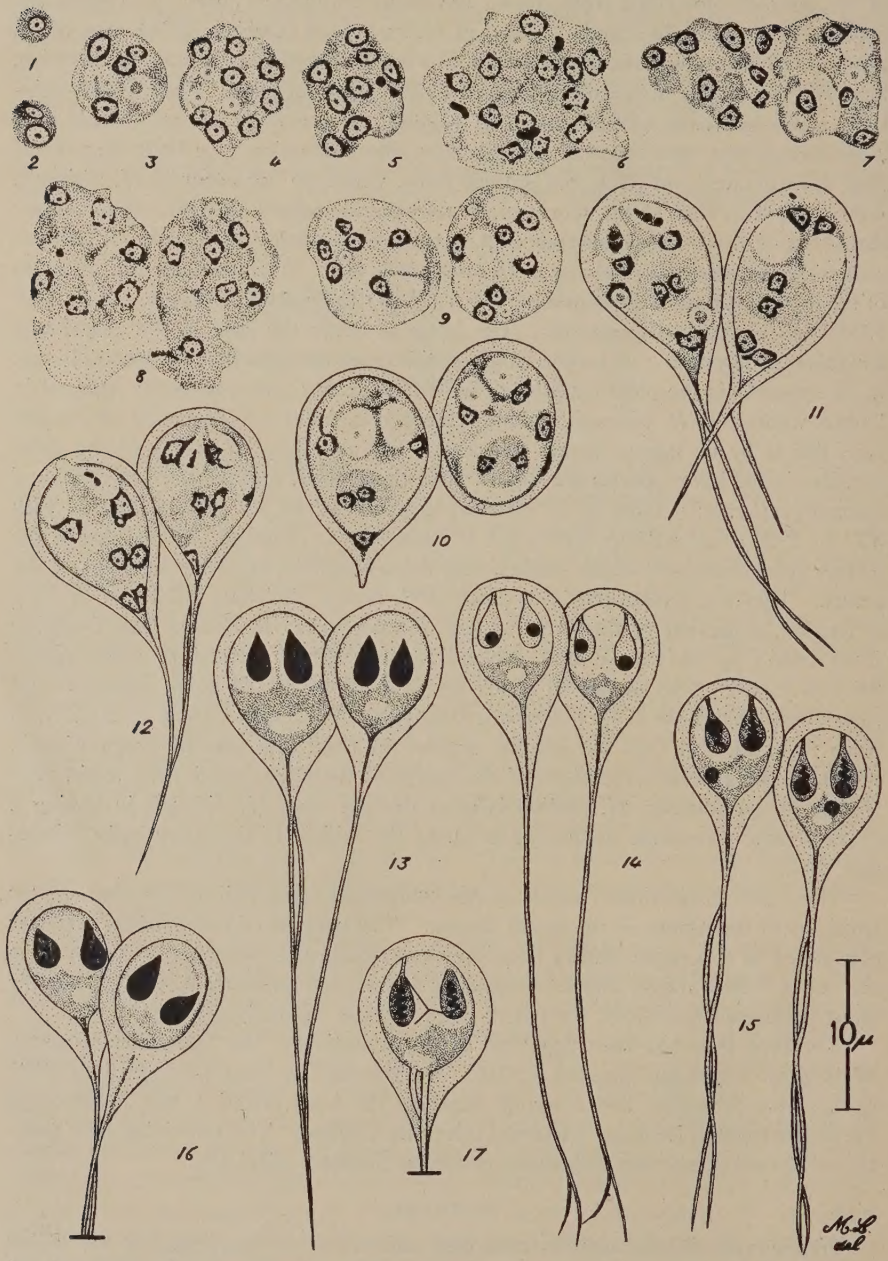
There are no previous records of MYXOSPORIDIA from Fiji, or for that matter from any of the islands of the South Pacific. The parasite of *Leiognathus fasciatus* considered in this paper differs from previously described members of its genus as set out in detail above, and is designated *Henneguya vitiensis* n.sp. Its specific name is derived from *Viti,* the native name for the Fijian islands.

The type slide has been deposited in the collection of the Dominion Museum, Wellington (catalogue number Z100). A paratype has been catalogued as Z101 in the same collection, and a second paratype has been placed in the collection of the Department of Zoology, Victoria University College. The remaining nine paratype slides are in my own collection (catalogue numbers MH1–9).

SUMMARY

Henneguya vitiensis n.sp. is described from 12 of 40 specimens of the Fijian marine fish *Leiognathus fasciatus* (Lacépède, 1803), the type locality being Nandi Bay, Viti Levu. The species is disporoblastic and shows autogamy. It resembles *Henneguya otolithi* Ganapati, 1938, in that the two spores derived from each pan-

PLATE I



Henneguya vitiensis n. sp., from *Leiognathus fasciatus* (Lacépède, 1803).

EXPLANATION OF PLATE I

All figures drawn at a magnification of ×2,400 from air-dried smears of heart blood fixed in absolute methyl alcohol and stained with Giemsa.

Henneguya vitiensis n. sp., from *Leiognathus fasciatus* (Lacépède, 1803).

FIGS. 1–5. Uni- to octonucleate pansporoblasts.

FIG. 6. Pansporoblast in which spore formation is commencing, with twelve karyosomatic generative nuclei and four somatic residual nuclei.

FIGS. 7 and 8. Early differentiation of spores.

FIG. 9. Developing spores, which have adopted a rounded outline and show the beginnings of the polar capsules.

FIG. 10. Spores with shell formed, and sporoplasm clearly differentiated. Example on left showing developing tail process(es).

FIGS. 11 and 12. Stages showing development of polar filaments and growth in length of tail processes.

FIGS. 13 and 16. Fully developed spores, showing usual densely stained appearance of polar capsules.

FIG. 14. Lightly stained spores, showing capsular nuclei.

FIG. 15. Lightly stained spores, showing capsular nuclei and synkaryon in sporoplasm.

FIG. 17. Lightly stained spore (tail processes not drawn) showing polar filaments and posterior extensions of spore cavity. A sister spore was present, but is not figured.

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sporoblast remain in association with one another, and in many morphological features of the spore, but has no trace of the transverse thickening at about the middle of the spore which is characteristic of the latter species. *H. vitiensis* n.sp. also has affinities with *Henneguya salminicola* Ward, 1919, but differs from this species in having markedly smaller polar capsules, in the total absence of sutural folds and in the fact that the tail processes are never significantly unequal in length.

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A NEW CESTODE, *RAILLIETINA (R.) MULTITESTICULATA* N. SP. FROM THE RED HOWLER MONKEY*

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Among parasites collected in 1925 by an expedition to British Guiana headed by Dr. H. E. Enders, was a bottle of cestodes which were recently made available to the writer for study. The bottle contained 162 worms in alcohol, all of the same species and recovered from the small intestine of a red howler monkey taken at the junction of the Mazaruni and Cuyuni Rivers in northern British Guiana. The cestode represents a new species of the subgenus *Raillietina* for which the name *Raillietina (R.) multitesticulata* is proposed.

The specimens, although well extended, were difficult to stain because of long preservation but from several series of whole mounts and sections the important morphological features could be determined. All figures were drawn with the aid of a microprojector.

Raillietina (R.) multitesticulata n. sp.
(Figs. 1–6)

Diagnosis: (All measurements in millimeters.) Length of complete specimens 270–330 (av. 300); maximum width 3.2; mature strobila with about 420 proglottids. Scolex diameter 0.39–0.57 (av. 0.547); suckers 0.120–0.153 in diameter and armed with 4–7 rows of hooks 0.008 long; lumen of suckers with prominent tissue plug. Diameter of rostellum 0.12–0.15 (av. 0.135). Rostellar hooks hammer-shaped, arranged in two rows; hooks of anterior row 0.015 long, of posterior row 0.017. Genital pores unilateral, sinistral; genital ducts pass between excretory vessels. Testes 0.06–0.08 in diameter and 115–120 in number, in two fields, one on each side of the ovary; more numerous on the aporal side; a small internal seminal vesicle present. Vagina expanded near ovary to form a seminal receptacle; ovary and vitelline gland slightly poral in position. Gravid proglottids with 47–80 thick walled uterine capsules, each containing 3–8 eggs 0.05–0.06 in diameter.

Host: Alouatta seniculus (Linn.) Allen, 1904.

Locality: Kongarooma, British Guiana, South America.

Type Material: Cotypes no. 46471, Helminthological collection, U. S. National Museum.

The strobila is widest at the level of the mature proglottids, and the breadth decreases gradually toward the gravid end where it is only about 2.0. All of the proglottids are broader than long except the fully gravid ones which are about as long as wide.

The scolex is set off from the neck by only a slight constriction. The acetabular hooks are apparently easily lost since they are partially lacking in several specimens. They do not form an annular pattern around the sucker opening, but arrangement in diagonal rows is evident. The spinose area encircling the sucker opening is widest anteriorly where seven hooks can be counted in a diagonal row. This area decreases in breadth posteriorly where there are only four hooks per diagonal row.

The bases of the posterior row of rosteller hooks are set back about two micra from those of the anterior row so that the hooks of both rows form an almost even single ring. The width of the neck is from 0.29–0.35. Segmentation begins 2.5–3.0 from the base of the scolex.

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* Study made under the direction of Professor R. M. Cable.

The genital primordium first becomes apparent at about the 34th segment, where it is merely a deeply staining area in the center of the proglottid. The primordia of the male and female genital ducts become distinct at about the 100th segment and in the 200th, the vagina, vas deferens, and cirrus sac are well developed, and the ovary and testes are appearing. The testes are well formed by the 220th and the ovary is apparent by the 230th segment. The ovary begins to disappear by the 290th segment and by the 300th it has disintegrated; here eggs are apparent among the testes, although a distinct uterus is not observed. In approximately the 350th segment, egg clusters have formed and there is no evidence of testes. The parenchyma around the egg clusters becomes progressively condensed until, by the 400th proglottid, a thick capsule encloses each cluster. There are usually about 20 such fully gravid proglottids in an entire worm.

The ventral excretory ducts are very conspicuous and measure up to 0.142 in diameter. The transverse ducts are somewhat narrower, though readily visible. The dorsal ducts, which are very difficult to find in mature and gravid proglottids, are very narrow, measuring about 0.015 in diameter and are medial to the ventral ducts. The longitudinal nerve is very inconspicuous and lies just lateral to the ventral ducts.

Mature segments are 0.69–1.00 long and 3.00–3.16 wide. The cirrus sac is relatively small, being 0.262–0.308 long, and very muscular; it crosses the nerve but does not reach the excretory vessels. The cirrus is unarmed, and when everted is always flexed at nearly a right angle near the distal tip. This suggests that self copulation, although not observed, may readily take place. The ejaculatory duct enlarges to form a small internal seminal vesicle and then passes through the wall of the cirrus sac to join the highly convoluted vas deferens which may be traced very nearly to the ovary before it receives the vasa efferentia.

The ovary is extremely lobed and semicircular in moderately extended proglottids, where it measures 0.345–0.4 in diameter. Directly behind and slightly ventral to it is the vitelline gland. The shell gland is located in the usual position and is very inconspicuous. The seminal duct connecting the small seminal receptacle to the female complex is very short. The vagina is thin-walled with the exception of approximately 0.3 at its distal end where it is very muscular. It opens posterior to the cirrus and the genital atrium is extremely shallow. The egg capsules measure 0.250–0.375 × 0.180–0.187 and their walls may be as thick as 0.03; they are distributed throughout the parenchyma, extending beyond the ventral excretory vessels. Hooks were not observed in the developing embryos, even in the oldest proglottids.

In the subgenus *Raillietina,* only three species have been reported which have more than 75 testes, and, of these, only one occurs in primates. This is *Raillietina* (*R.*) *demerariensis* (Daniels, 1895) reported from man in British Guiana. This species has fewer testes (60–86) and a larger number of uterine capsules (120–150) than has *R. multitesticulata.* The other two species which resemble *R. multitesticulata* are *R. leptacantha* (Fuhrmann, 1908) from galliformes in Brazil, with 80–86 testes and a much smaller cirrus sac (0.066–0.080) and *R. struthionis* (Houtuyn, 1773)which occurs in Struthioniformes, and is a larger species with 150–200 testes.

ADDENDUM

The description of *R.* (*R.*) *alouattae* Baylis, 1947 (Jour. Linn. Soc. London, 41: 406–414), was not seen until the present paper was in press. This species from the black howler monkey in Dutch Guiana is very similar to that here described but discrepancies in the following respects indicate that the two are distinct: maximum body width, number of rows of acetabular hooks and their distribution, position of the genital pore, size of the cirrus, confluence of testes between poral and aporal groups, number of uterine capsules and the number of eggs per capsule.

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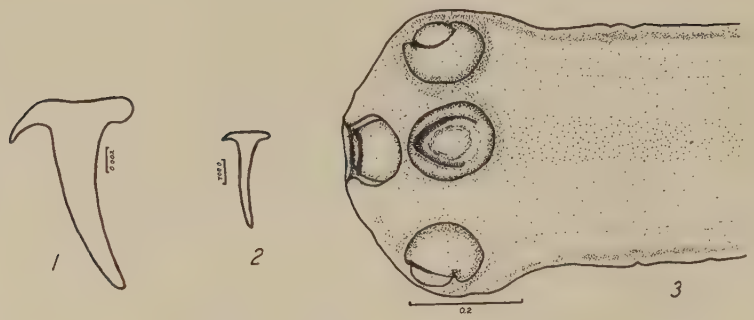
EXPLANATION OF FIGURES

(All figures concern *Raillietina* (*R.*) *multitesticulata*)

1. Rostellar hook.
2. Acetabular hook.
3. Scolex.
4. Mature proglottid.
5. Proglottid showing egg masses before their encapsulation.
6. Gravid proglottid with uterine capsules.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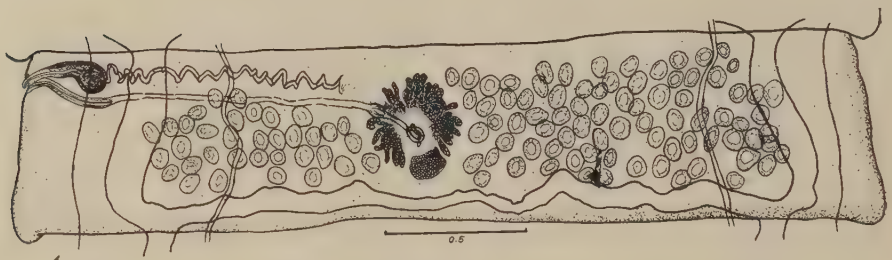
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A NEW SPECIES OF *TRIGANODISTOMUM* (TREMATODA: LISSORCHIIDAE) FROM THE SACRAMENTO SUCKER, *CATOSTOMUS OCCIDENTALIS* AYRES.

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In 1948 during a preliminary survey of the parasites of fishes in Clear Lake, Lake County, California, (Haderlie, manuscript thesis) nineteen mature suckers were examined for intestinal helminths. In addition to other parasites found, five of these fish carried from one to thirteen flukes. The flukes were stained in borax carmine and mounted in clarite. A study of these permanent mounts revealed that the flukes possessed characteristics of two genera of trematodes previously found in fish closely related to the Sacramento sucker.

In 1917, Magath described a new fluke from the intestinal tract of the buffalofish, *Ictiobus*. He set up a new genus, *Lissorchis,* and named this fluke *Lissorchis fairporti*. He found that this new genus could not be embraced by any of the previously described subfamilies and designated it as the type of a new subfamily Lissorchiinae in the family Plagiorchiidae. McMullen (1937) revised the taxonomy of this family and raised the Lissorchiinae to family rank.

Simer (1929) erected the genus *Triganodistomum* to include a single species from the gut of *Ictiobus* which he described under the name *Triganodistomum translucens*. He compared this genus with some closely related forms, but made no comparisons with the genus *Lissorchis* of Magath. He assigned *Triganodistomum* to the family Plagiorchiidae.

Van Cleave and Mueller (1932, 1934) made an extensive study of fish trematodes which bear lateral genital pores. They concluded that *Plagioporus, Plagiocirrus, Triganodistomum* and *Lissorchis* are all closely related, that these four genera represented but a single family, and all of them were assigned to the Allocreadiidae. In 1932, Van Cleave and Mueller described two new species of the genus *Triganodistomum, T. attenuatum* and *T. simeri,* both from the digestive tract of the common sucker of Oneida Lake.

Cort (1918) described a new larval trematode, *Cercariaeum mutabile,* from planorbid snails of Douglas Lake, Michigan. Cort suggested the relationship of this larva to the subfamily Allocreadiinae. The life history of this form was determined by Wallace (1939). He found that the tailless larvae developed to maturity in the chub sucker and that the adult trematodes belonged to the genus *Triganodistomum*. The adult trematode thus became *Triganodistomum mutabile*. Contrary to Cort (1918) and Van Cleave and Mueller (1932, 1934), Wallace (1941), on the basis of morphological and embryological similarities, placed *T. mutabile* tentatively in the family Lissorchiidae. Hopkins (1934) had excluded the genus *Triganodistomum* from the family Allocreadiidae by reporting that forms having ophthalmoxiphidiocercariae should form the basis of the family Allocreadiidae, and Cable and Hunninen (1942) agreed with this opinion. This

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paper, therefore, follows Wallace (1941) and places *Triganodistomum* in the family LISSORCHIIDAE.

In 1942, Fischthal described *Triganodistomum hypentelii* from the hog sucker of the Saline River in Michigan, and gave a key to the species of the genus *Triganodistomum.*

The trematode from the Sacramento sucker has a lateral genital pore and other characteristics which fit it into the family LISSORCHIIDAE. It possesses characteristics of both the genus *Lissorchis* and the genus *Triganodistomum,* but resembles the latter more closely, differing from the other members of the genus *Triganodistomum* mainly in the shape of the ovary. It is therefore proposed to name this trematode *Triganodistomum polylobatum* n. sp.

Mueller (1934) has given an emended generic diagnosis of the genus *Triganodistomum.* If this is revised by the addition of the words "or more" to the description of the ovary, so that the conditions of the ovary reads: "Ovary with three or more distinct lobes . . . ," the genus will accommodate the trematode described here as new.

Triganodistomum polylobatum n. sp. differs from *Lissorchis fairporti* in the following respects: (1) The cuticle has spines around the suckers and the full length of the ventral surface, especially prominent near the acetabulum. The dorsal surface is smooth. *L. fairporti* has spines over the entire cuticle, especially prominent at the posterior end. (2) The testes are fairly round in *T. polylobatum,* whereas *L. fairporti* has oblong testes. (3) The relative size of the suckers differs. (4) The distribution of the vitellaria is different in the two.

T. polylobatum resembles *L. fairporti* in overall size and shape, size and form of the ovary, and in the position of the testes.

T. polylobatum differs from *T. attenuatum* mainly in the shape of the ovary. The ovary in the former is definitely follicular with usually 4 to 6 lobes; the latter has a trilobed ovary. The testes in *T. polylobatum* are located farther posteriorly than the testes in the other fluke, and the acetabulum of the new fluke is nearer the middle of the body than it is in *T. attenuatum.* The overall size of the two flukes is different. However, the two are alike in the nature and distribution of the vitellaria, the shape and size of the testes, and the size and shape of the suckers.

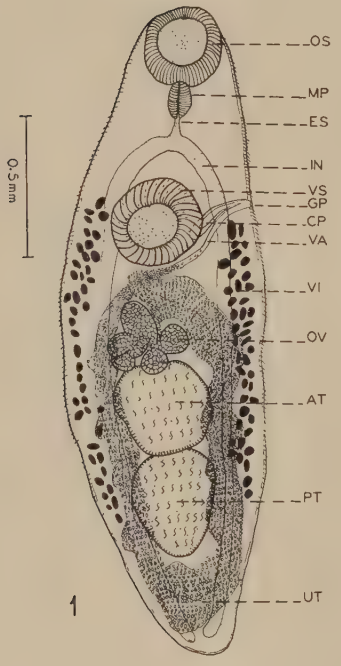
EXPLANATION OF PLATE

All drawings were made with a camera lucida from permanent mounts stained in borax carmine and mounted in Clarite.

Symbols

AT	anterior testis	OV	ovary
CP	cirrus pouch	PT	posterior testis
EG	eggs	SI	spiny integument
ES	esophagus	UT	uterus
GP	genital pore	VA	vagina
IN	intestinal crurum	VD	vitelline ducts
MP	muscular pharynx	VI	vitellaria
OL	ovarian lobes	VS	ventral sucker
OS	oral sucker		

FIG. 1. Ventral view of *Triganodistomum polylobatum.*
FIG. 2. Lateral view showing characteristic multi-lobed appearance of the ovary.
FIG. 3. Enlarged view of ovary and anterior testis.
FIG. 4. Enlarged view of the genital pore region.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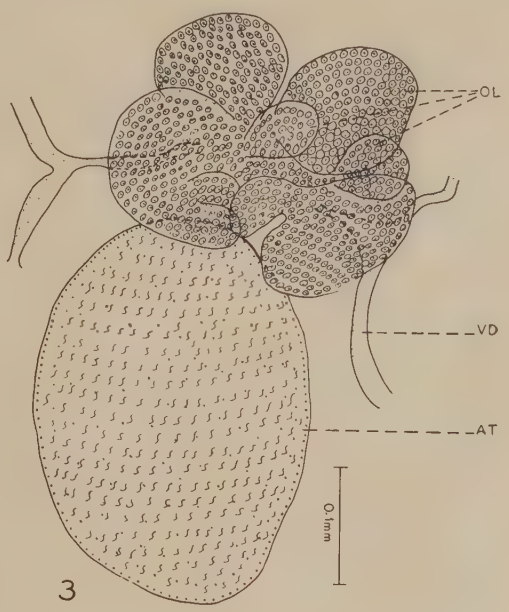
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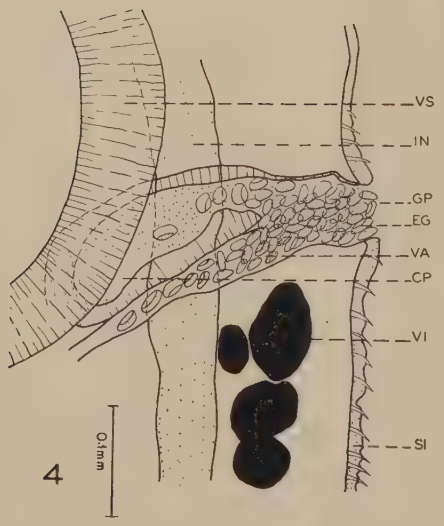
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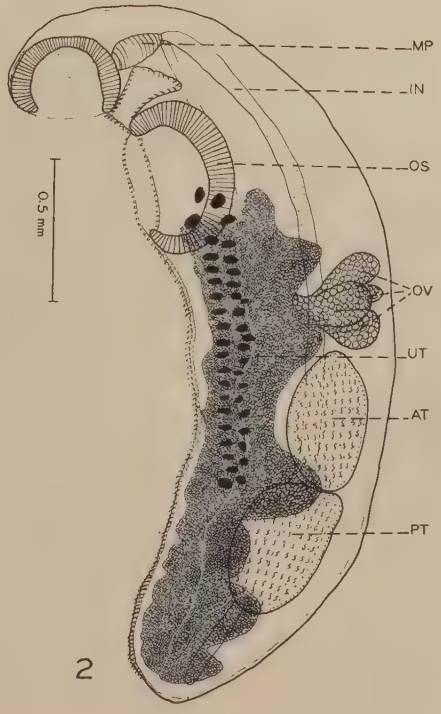
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T. polylobatum differs from *T. mutabile* in size, the latter being much smaller; and differs from *T. hypentelii* in the distribution of the vitellaria. It differs from both these forms in the shape of the ovary.

Triganodistomum polylobatum n. sp.

Description: With the characters of the genus *Triganodistomum* as emended by Mueller (1934) and revised above:

Body flattened, elongate, tapering posteriorly. Average length 2.1 mm. Average maximum width 0.7 mm. Suckers prominent and well developed, oral sucker averages 0.28 mm in diameter, ventral sucker 0.32 mm. Acetabulum located in middle third of body. Genital pore sinstral, at the level of the middle of the acetabulum. Cirrus sac overlaps acetabulum dorsally. No spination of the cirrus evident. Ovary composed of 4 to 6 fairly large and distinct follicles or lobes. Ovary 0.28 mm. in diameter, 0.3 mm. posterior to the acetabulum. Testes fairly round or oval in shape, 0.28 mm. average diameter. Ovary overlaps anterior testis a little. Posterior limits of testes 0.26 mm. from posterior tip of fluke. No pre-pharynx. Pharynx muscular, 0.1 mm. maximum diameter, leads into short (0.03 mm.) esophagus. Intestinal crura run to within 0.07 mm. of posterior end of worm. Vitellaria clear and distinct, follicular, extending along the lateral areas from the middle of the acetabulum to the middle of the posterior testis. Transverse yolk ducts on each side give rise to common ducts at the level of the ovary. Uterus extends from genital pore to the posterior tip of the fluke, filled with small (0.017×0.025 mm.) eggs. The cuticle is covered with spines on the ventral surface, the largest spines near the acetabulum. Small spines around suckers and dorso-lateral line to the level of the testes.

Type host: Catostomus occidentalis Ayres (Sacramento sucker) from Clear Lake, Lake County, California.

Intermediate hosts and developmental stages unknown.

Holotype and paratypes deposited in U. S. National Museum. Series of paratypes in the collection of the Department of Zoology, University of California, Berkeley, California, and in the author's collection.

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THE MOUTH PARTS OF THE ADULT FEMALE TROPICAL RAT MITE, *BDELLONYSSUS BACOTI* (HIRST, 1913) FONSECA, 1941 [= *LIPONISSUS BACOTI* (HIRST)], WITH OBSERVATIONS ON THE FEEDING MECHANISM[1]

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INTRODUCTION

The role of the tropical rat mite, *Bdellonyssus bacoti* (Hirst, 1913), as a vector of a variety of diseases of man and animals has recently received a great deal of attention. In the course of a study of the transmission to the cotton rat of the filarial worm, *Litomosoides carinii* (Travassos, 1919), a knowledge of the feeding mechanism of the mite has been necessary. To understand this mechanism a detailed study of the mouth parts of the mite became imperative. The results of this study were reported to the American Society of Parasitologists in December 1949 and an abstract of the work appears in the supplement of the Journal of Parasitology for 1949. While the paper was undergoing editing for the Journal of Parasitology, Hughes (1949) published his findings on the same mite. Through the kindness of Dr. Stunkard the present paper was recalled for the purpose of incorporating a review of Hughes' work.

The author wishes to express her indebtedness to Dr. Don W. Micks, the University of Texas, for his suggestions and encouragement throughout the progress of this investigation. The advice of Mr. R. E. Snodgrass and Dr. E. W. Baker on points of terminology and interpretation is acknowledged with appreciation. Grateful acknowledgment is made to Dr. G. W. Wharton of Duke University for his suggestions and help during the revision of the paper.

MATERIALS AND METHODS

The mouth parts proved to be so complex and so compactly arranged that several approaches to their study were necessary. Some features were best revealed by whole mounts, some by serial sections and some by teasing and manipulating the structures under the microscope.

With few exceptions the standard methods of histological technics were employed. Prior to sectioning, mites were immersed in boiling 70 per cent isopropyl alcohol and allowed to remain in the alcohol from two to four weeks. It was also found that embedding in paraffin (60°–62°) directly from xylol was preferred to the use of an intermediate xylol-paraffin mixture. Sections, ranging from 5 to 17 micra, were stained in a 95 per cent alcoholic solution of phloxin and destained ten minutes in each of two changes of 95 per cent alcohol.

Living mites could be studied by placing the mites dorsal side down on a piece

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[1] A contribution from the Department of Preventive Medicine and Public Health, University of Texas School of Medicine, Galveston, Texas. The work on which this paper is based was part of a program under the direction of Dr. J. Allen Scott and supported by a Research Contract with the Office of Naval Research.

of scotch tape which had been fixed to a slide. Both hands can thus be employed to probe the individual mouth parts under a dissecting microscope as well as under a 16 mm. compound objective.

Mites killed in 70 per cent alcohol were used to study the isolated parts. After immersion in alcohol for one to two weeks, they were mounted in a drop of Berlese's medium or a mixture of polyvinyl alcohol, lactic acid and phenol (Downs, 1943). Before the cover slip was added to the preparation, the mite was bisected between the first and second pair of legs. The posterior portion was removed from the preparation in order to keep the slide as clean as possible. The anterior portion was held in position by inserting a fine needle (*Minute Nadel*) through the cut edge into the muscles of the pharynx. A second needle was then used to gently separate the sclerotized regions from the muscular attachments as well as from each other. When the desired members were isolated, a cover slip was placed on the preparation. By sliding the cover slip back and forth on the preparation before it dried, the true contour of the parts as well as their actual relationships to each other could be studied.

MORPHOLOGY

In most ACARINA there is no distinct line of demarcation between head, thorax and abdomen. The gnathosoma or capitulum, however, is easily differentiated by its attachment to the ventro-cephalic surface of the idosoma or body of the mite by a belt of membranous tissue and is movably articulated with the body (Plate I figs. 1–2, C.A.). The general form of the capitulum can be likened to a cylinder supporting a cone. The cylinder is the basis capituli. The cone is the beak (rostrum of Hughes). Laterally, flanking the beak on either side, the basis capituli supports a pair of segmented palpi (Plate I fig. 2, P.).

Basis Capituli

The basis capituli is formed by the fusion of the coxae of the palps and possibly by the ventral portion of the anterior segments. In many mites the lateral walls of the basis capituli do not extend dorsally over the chelicerae but in the MESOSTIGMATA, the group to which *B. bacoti* belongs, these lateral walls extend dorsally and fuse medially to form a sclerotized ring which forms the outer sclerotized protection of the basis capituli. In the mid-ventral region there is a groove, the capitular groove; internally, just dorsal to the capitular groove is the pharynx and above the pharynx is the epistome which forms the floor of the cheliceral sheaths in which the chelicerae glide (Plate I fig. 1).

Beak

The structures comprising the beak are formed by various anterior projections from the basis capituli. The ventral wall of the basis capituli and the ventro-lateral walls of the pharynx project anteriorly to form the hypostome. The dorsal wall of the pharynx and the ventral surface of the epistome extend anteriorly to form the epipharynx. The labrum, located immediately dorsal to the epipharynx, is an anterior projection of the dorsal surface of the epistome. The dorsal surface of the basis capituli extends anteriorly to form the dorsal covering or tectum of the beak (Plate I fig. 1; Plate III fig. 17).

Palps

The palps, one on either side of the beak, are freely movable on the antero-lateral angles of the basis capituli (Plate I fig. 2). Each palp consists of six segments (Plate II fig. 10) of which the trochanter or first movable segment is the largest in diameter and its length is about equal to its breadth. The medial surface of this segment has a sclerotic flap (F) which is in close association with the lateral sides of the beak. The third and fourth segments (femur and genu) differ little from the second except that they are slightly smaller. The fifth segment (tibia) contains a cup-like depression from which arises the sixth segment. Projecting from the base of the tarsus is a large, forked hair. The sixth segment is the smallest of the palpal segments. It is club shaped and contains distally a circular pad of setae which vary in length. Hughes represented the forked hair, found on the tarsal segment of the palps, as arising from the fifth segment or tibia of the palp which is incorrect.

The arrangement of the setae on the six segments varies little from one mite to another, but the individual setae do differ both in thickness and in length. That the setae of the palps are of a highly tactile nature is apparent from observing the action of the palps while a mite is in motion or feeding.

Within the second segment of each palp is found a cellular mass (Slv.) capable of elongating or contracting. It is usually seen in the second and third segments (Plate I fig. 2) but has been observed to extend as far as the fifth segment and also into the beak. Neither Hughes (1949) nor Stanley (1931), the latter in his work on *Laelaps echidninus,* mentioned the salivary glands as such or exactly where they are to be found, but both state that salivary ducts enter the "styli" and open outside through a pore. It would seem that these structures in *B. bacoti* are the salivary glands, although no distinct duct or connection was found in the sections studied. Killed and fixed mites have not revealed the histological structure of the cellular material, but in living mites it appears to be similar to the tissue of the malphigian tubules.

Capitular groove

The medial ventral surface of the basis capituli is modified into a shallow groove, the capitular groove (Plate I fig. 2; Plate IV figs. 25–33). The floor of the capitular groove contains nine to ten, usually nine, small, wedge-shaped teeth directed anteriorly (Ct.T.). The walls of the capitular groove along with the ventral surface of the basis capituli extend into the beak where they form the ventral surface of the hypostome. The convex sides of the capitular groove meet anterior to the hypostomal groove as a forked prong (Plate I fig. 2; Plate II figs. 4–5, Li.G., C.S.Li.G.). A pair of setae flank the groove at the middle of the basis capituli. The capitular groove might well represent the deutosternum. Hughes (1949) referred to this modification as the hypostome.

Tritosternum

A structure associated with the capitulum but actually separate from it is a bifid process or tritosternum. It is attached to the mid-ventral portion of the belt of membranous tissue supporting the capitulum and is the first structure observed from a ventral view of a mite (Plate I figs. 2–3, B.B.).

The process is freely movable and by careful manipulation can be removed from its attachment for study. Its removal usually carries with it the sternal plate (Plate I fig. 3, St.P.). The anterior portion of the tritosternum normally lies in the capitular groove.

It is divided into three regions: (1) the base (B.B.), that region attached to the body of the mite; (2) the neck (N) and (3) the blade-like processes or lacinae (B-L.P.). The basal portion narrows sharply to form the neck which is almost twice the length of the base. Beyond the neck the structure bifurcates forming the two lacinae. These two processes have their thickest diameter at their points of origin, becoming very slender and pointed at their tips. Setules are present on the blades through their entire length.

Extending laterally along the base and to the tip of the blades is a thin, transparent membrane (M). At the tip of the blades it is difficult to distinguish the membrane from the blades. The membrane is widest at the neck region and its edges here are serrated. This toothed appearance has no consistent pattern among mites, in fact, opposite sides belonging to the same structure can differ. The tritosternum is not a solid structure but contains a lumen which begins blindly at the base and terminates in the lacinae (Plate III fig. 24, B-L.P.; Plate IV figs. 28–34, N., B.B.).

Chelicerae

The slender, three-segmented chelicerae occupy the dorsal region of the basis capituli and their free, distal extremities protrude anteriorly above the labrum. Hughes (1949) has a very detailed and excellent description of the chelicerae and the cheliceral sheaths so that it will not be repeated here. Hughes, however, described "a thin membranous hood" as covering the fixed digit of the chelicerae. While such a hood has been observed in the immature stages of development of *B. bacoti,* no such hood has ever been observed in the female adult.

The retraction of the chelicerae is made possible by stout muscles which are inserted on the posterior extremities of the shafts. These retractor muscles pass backward and upward and are attached to the dorsal wall of the propodosomal region. Protraction of the chelicerae would appear to be effected by a method similar to that described for *Argas persicus* by Robinson and Davidson (1913). A general contraction of the dorso-ventral body muscles apparently creates a pressure within the body cavity which thrusts the chelicerae out through their sheaths. Further movement of the chelicerae is stopped by the action of the retractor muscles.

Epistome (subcheliceral plate of Hughes)

The epistome extends throughout the length of the basis capituli. In sagittal section it can be seen to lie between the dorso-lateral dilator muscles of the pharynx and the shafts of the chelicerae (Plate I figs. 1, S.E.P.). It is a structure which has been described in both mites and ticks under a variety of names. These names such as epistomal plate, sub-cheliceral plate and sub-cheliceral epistomal plate have been discussed at length by Snodgrass (1948). It is bifurcated except at its anterior end, *i.e.,* at the junction of the basis capituli and the beak where it is supported laterally by stout, sclerotized bars extending from the ventral surface of the capitulum. These lateral bars also serve to separate the palps from the beak. Plate III fig. 23).

In cross section the anterior surface of the epistome resembles a triangle and extends forward as the labrum (Plate III figs. 17–22, V.). Just posterior to the labrum at the junction of the basis capituli and the beak, the ventral surface of the epistome curves ventrally and fuses with the dorso-lateral sides of the pharynx. The aperture formed by this sclerotic ring leads into the epipharynx (Plate III figs. 22–23, L.A.). The bifurcated portion at its posterior end curves ventrally and serves as the origin for three pairs of longitudinal extensor muscles (Plate I fig. 1; Plate IV figs. 32–33, L.E.).

Pharynx

The pharynx occupies the ventral portion of the internal cavity of the basis capituli (Plate I fig. 1) and consists of a tube of triangular cross section (Plate IV figs. 26–34). At its anterior end the pharynx bends ventrally and its ventral edge fuses with the ventral wall of the capitulum at the junction of the beak and the basis capituli (Plate III fig. 23, V.L.Ph.). At this junction, also, the dorso-lateral edges of the pharynx fuse with the epistome. Its lateral walls spread outward and extend anteriorly to become the hypostome.

Pharyngeal muscles

The musculature of the pharynx of the adult of *B. bacoti* is nearly identical with that of *Laelaps echidninus* described by Stanley (1931). The following description is to a large extent adapted from that of Stanley. The peristaltic-like movement of the pharynx is brought about by the action of three sets of constrictor muscles (Plate IV figs. 26–32, V.L.,D.), and four sets of dilator muscles (V.L.D., D.L.D.). The three sets of constrictors are alike in form, each muscle being attached at the edges of the pharyngeal walls and on contraction cause the sides of the triangular tube to buckle inward along the middle line. The two ventro-lateral sets (V.L.) are arranged directly opposite to each other and consist of seven muscles arranged serially, while the dorsal set (D) has only six muscles. The two ventro-lateral sets of dilator muscles (Plate III figs. 23–24; Plate IV figs. 25–33, V.L.D.) have their origin on the ventro-lateral walls of the capitulum and their insertion on the ventro-lateral walls of the pharynx, thus working in opposition to the constrictors (V.L.). The two dorso-lateral sets of dilators (D.L.D.), numbering five muscles each, have their insertion on the dorsal wall of the pharyngeal tube, but the individual muscles divide themselves into groups in the matter of origin. The two anterior muscles of each set have their origin on the ventro-lateral surfaces of the outer epistome (Plate IV figs. 26–27). The three posterior muscles of each set originates from the lateral walls of the capitulum (Plate IV figs. 28–32). The anterior region of the pharynx is here referred to as the pre-pharynx since the musculature of the pre-pharyngeal region differs from that of the remainder of the pharynx in that the dorsal constrictors and dorso-lateral dilators are absent from its dorsal surface. These are replaced by one of three pairs of extensor muscles which originate from the posterior end of the epistome.

Hughes described only four muscles for each set of constrictors. Although no study has yet been made on the immature forms of *B. bacoti*, it is highly possible that in the larval or nymphal stages there is a reduced number of pharyngeal muscles. Hughes did not specify that he was dealing exclusively with the adult.

Longitudinal extensors

As mentioned above, the posterior curved portion of the epistome serves for the origin of three pairs of longitudinal extensor muscles (Plate IV figs. 31, 33). One pair (A) originates from the medial surfaces of the epistome; a second pair (B) originates from the ventral surfaces of the epistome, while the third pair (C) originates partly from the ventral surfaces and partly from the lateral surfaces of the epistome. These muscles extend forward and divide at the place of insertion. Two pair enter the aperture of the epipharynx and insert on its inner sclerotized shell. The outer pair inserts on the dorsal wall of the pre-pharynx (Plate I fig. 1, E.P-ph., E.L.; Plate IV fig. 25).

Hughes described these muscles as five in number and referred to them as the "cheliceral protractor muscles." He stated that they "have their insertion on the cheliceral sheaths where these merge with the cheliceral shafts" and that they arise "within the base of the epipharynx." As to their function he concluded that "these muscles on contraction will pull the chelicerae forward and protrude them beyond the tip of the rostrum" and that "apart from protracting the chelicerae, these muscles will have a secondary effect on the epipharynx. Since they are attached to its ventral wall, anterior to the thick anterior edge of the subcheliceral shelf, their contraction will tend to raise this floor and to depress the tip of the epipharynx into the hypostomal groove. Since the chelicerae are freely movable and the base of the epipharynx is relatively fixed, their contraction can only result in the contraction of the chelicerae."

As mentioned above, the muscles actually originate from the epistome and not from the cheliceral sheaths. For this reason alone it is obvious that Hughes' unorthodox views concerning the function of the anterior longitudinal extensors are incorrect. Even if the muscles actually did attach to the cheliceral sheaths they would act as retractors and not protractors since they would have to bend anteriorly dorsal to the epistome when the chelicerae were extended. Variable pressure in the haemocoel is responsible for movement of appendages in other Acarina; for example, Brown (1949) reported that there are no intrinsic extensors in the palp of *Trombicula alfreddugèsi* (Oudemans, 1910).

Hypostome

The hypostome from a ventral view appears as two triangular plates, each bearing three hypostomal setae (Plate I fig. 2, T.P.Li.). It is derived from the ventral sides of the basis capituli extending anteriorly (Plate II figs. 4–5) and from the anterior extensions of the ventro-lateral walls of the pharynx (Plate III figs. 21–22, V.L.Ph.). The exposed ridges of these ventro-lateral walls are modified into two rows of teeth (Plate II figs. 4–5, 7, Hst.T.). The gutter formed by the walls of the hypostome is the floor of the oral channel. Dilator muscles similar to the ventro-lateral dilator muscles of the pharynx originate from the ventral surface and insert on the hypostomal teeth (Plate III figs. 15–21, D.M.Hst.).

Mala externa and mala interna

These lateral walls of the beak have been described in some detail by Hughes and will be considered only briefly here. More ventral and passing without clear boundarise into the hypostome is the mala externa Plate I fig. 2; Plate II figs. 4–5; Plate III

figs. 13–15, I-Cxp.). It resembles a parabola when flattened from which extends anteriorly a sclerotized rod supporting a leaf-shape, grooved stylus, referred to as the "flange" by Hughes (Plate II figs. 4–5, R., S.I-Cxp.). Dorsal to the mala externa but continuous with it proximally is the mala interna (Plate III figs. 14–17, II-Cxp.). The latter is a large, lanceolate, hollow process which flares dorsally and medially so that it encloses the other mouth parts and "becomes closely applied to the cheliceral shafts when these are protracted" as described by Hughes (Plate II figs. 4–5, 7; Plate III fig. 17). The tapering distal ends of the mala externa and the mala interna remain in close association with each other. The ventro-median edge of each mala interna is slightly grooved and the latero-dorsal edge of the mala externa, fitting into this groove, loosely locks the two processes together (Plate III fig. 13).

Epipharynx

The epipharynx, a hollow, four sided, pyramidal structure with a circular base occupies the most central position of the beak structures (Plate II fig. 6). It appears in cross section as a diamond shaped structure between the hypostome and the labrum (Plate III figs. 14–21; Plate I fig. 1, L.). At its base the epipharynx becomes nearly circular and is supported by the cuticular ring formed by the fusion of the dorso-lateral edges of the pharynx with the anterior, ventral surface of the epistome (Plate III figs. 21–24). Except at its base the ventro-lateral sides of the epipharynx lie in the furrow formed by the ventro-lateral sides of the hypostome (Plate III figs. 14–20).

The ventral edge of the epipharynx has a rod-like thickening (L.R.) which apparently lends rigidity to the entire sturucture. Its dorsal edge is characterized by a fin-like extension (F.E.). Each lateral edge of the epipharynx is modified into teeth, one row anteriorly and two rows posteriorly (Plate III fig. 17). In the region where the four-sided shape is converging into the circular form of the base, the teeth along these lateral edges articulate with the toothed ridges of the medial surfaces of the styli (Plate III figs. 19–20).

Entering the aperture of the epipharynx and projecting into its lumen, are four of the six bands of longitudinal extensor muscles originating on the ventral, medial and lateral surfaces of the epistome (Plate III figs. 23–24, L.E.). These four muscles insert on the inner surface of the shell of the epipharynx in the region of the fin-like extension (Plate I fig. 1). As already mentioned, the remaining muscles of this longitudinal group are inserted on the dorsal wall of the pre-pharynx.

Labrum

The labrum was fully discussed by Hughes and will not be considered here to any great extent. It is supported anteriorly by the epistome and forms the roof of the oral channel, lying between the chelicerae and the epipharynx. It is wedge-shaped consisting of a median, hollow, paralabra internum which follows the contours of the lateral wall of the epipharynx and a paralabra externum which arches away from it as a thin, sclerotized lamina with vein-like markings on its internal surface (Plate II fig. 8, C.P., M.E.).

While both the epipharynx and the labrum are hollow, it is unlikely that both contain the haemocoel of the gnathosoma as described by Hughes. There is no

doubt that the haemocoel is continuous into the cavity of the epipharynx, but it is hardly possible for it to extend into that of the labrum since the labrum is an anterior extension of the epistome, a solid apodeme (Plate I fig. 1, S.E.P., V.; Plate III figs. 20–23, V., S.E.P.).

Stylets

Two stylets, one on either side of the labrum, are always observed upon isolation of the labrum. They are the smallest of the beak structures and appear as slender, sclerotized bars (Plate II fig. 11). Apparently, they are always lost in the process of sectioning or staining since they have not been observed in any cross section. They were not mentioned by Hughes.

Styles

Laterally and immediately below the chelicerae lie a pair of long slender styles (Plate II fig. 12), which are supported basally on the medial walls of the palpal coxae at the level of the epistome (Plate III figs. 19–20, B.Max.). The medial surface of each base articulates with the lateral edges of the epipharynx. Hughes has homologized them with the "styli" of *Laelaps echidninus* as described by Stanley (1931). Stanley described the styli as receiving the salivary duct which "it follows to the tip, opening to the outside through a small pore." From the figures presented by Stanley, the "styli" as found in *L. echidninus* appear to be the mala interna of *B. bacoti.*

Tectum capituli

The tectum capituli or tectum (epistome of Hughes) forms a dorsal covering for the structures of the beak region. It is the anterior extension of the dorsal surface of the basis capituli (Plate I fig. 1; Plate III figs. 13–21, Tect.).

OBSERVATIONS ON THE FEEDING MECHANISM

A number of mites were individually observed under a dissecting binocular microscope while feeding on rats from 2 to 6 days old. The rats were fastened to a board, the hair removed from the abdomen with a depilatory and a single adult mite placed on each rat within a ring of cup grease. The mites had all been fed previously as adults but had been subsequently starved for at least two weeks. They began to feed sooner if the heat from an electric bulb were allowed to fall on the area. Under these conditions, three mites began to feed 16, 20 and 21 minutes, respectively, after being placed on the skin. Another fed only after 2 hours, while others did not feed within a period of 3 hours, after which time they were removed. They usually were observed feeding where a blood vessel could be seen beneath the skin.

If the skin were scarified, feeding began sooner—two mites beginning to feed 4 minutes after being placed on the skin. Occasionally, the mites appeared to begin feeding but detached themselves before blood was observed in the gut. Usually, however, blood could be seen to enter the gut soon after they attached. After this appearance of blood in the gut, 4 mites were completely engorged in 6, 7, 16 and 17 minutes respectively. Other mites did not become engorged but ceased to feed after 1 to 5 minutes and made no further attempt to feed. One of these, which had been feeding on intact skin for 5 minutes, failed to feed again during the next 10 minutes. A scratch was then made in the skin and about 15 minutes later this mite began

feeding at the point of the scratch and became fully engorged in 7 minutes. Usually, when a mite has begun to feed, jarring, pulling on the rat's skin or lightly touching the mite will not cause it to cease its blood meal. When fully engorged the mites detached abruptly and moved quickly away, rapidly protracting and retracting their chelicerae, rubbing their palps along their cheliceral shafts.

While feeding, the mite's body is elevated at an angle of approximately 45°; the second and third pair of legs are firmly attached and the fourth pair are barely able to touch the skin. The first pair of legs are either curved and adhering to the skin by means of their pulvilli or are waving about, repeatedly tapping the skin. The palps are also arched over the outer skin with their ends continually tapping the skin. The chelicerae are apparently the only organs which penetrate the skin.

From a study of the musculature and structure of the oral channel and pharynx, the following sequence of events is proposed as a possible mechanism of feeding. The author acknowledges the advice of Dr. R. F. Blount of the Department of Anatomy, University of Texas for his assistance in interpreting the action of the musculature.

The process of feeding by which the blood reaches the gut of a mite is accomplished in two phases. In the first phase the blood enters the pre-pharynx and pharynx by way of the oral opening, oral channel and the pharyngeal orifice. In the second phase the blood moves on through the oesophagus to the gut (Plate I fig. 1; Plate III figs. 19–24; Plate IV figs. 25–34).

The first or sucking phase begins with the protraction and retraction of the chelicerae to pierce the skin of the host, thus forming a pool of blood from which the mite feeds. The blood is drawn inward first by the enlargement of the oral channel (O.C.) by contraction of the dilator muscles of the hypostome (D.M.Hst.). The dilator muscles of the pre-pharynx (V.L.D.) and pharynx (V.L.D.) then contract enlarging those chambers and the negative pressure within these respective chambers sucks the blood into the pharynx. During this phase the longitudinal extensor muscles of the pre-pharynx (E.P-Ph.) are also contracted, serving to lift the dorsal wall of the pre-pharynx and the ventral wall of the base of the epipharynx (L.B.). Thus, the epipharynx (L.) is drawn upward against the labrum leaving the oral channel open.

The second or propulsive phase begins with the relaxation of the longitudinal extensors of the pre-pharynx (E.P-Ph.), followed by a contraction of the longitudinal extensors of the epipharynx (E.L.). This contraction produces a change in position of the epipharynx and a possible change in its shape. As a result of contraction, the epipharynx tips forward obstructing the oral channel and blocking the pharyngeal orifice. The oral channel is simultaneously reduced by a relaxation of the dilator muscles of the hypostome (D.M.Hst.) so that the hypostomal teeth (Hst. T.) approach the teeth of the epipharynx (L.T.). Consequently, the oral channel is closed momentarily preventing the blood from being regurgitated. At the same time, the dilators of the pre-pharynx and pharynx relax. The constrictors (D., V.L.) begin a wave of contraction and propel the blood from the pre-pharynx along the pharyngeal tube through the oesophagus to the gut. Just prior to the entrance from the pharynx into the oesophagus, the pharynx makes a dorsal loop and lacks dilator muscles. The dorsal loop and the constriction caused by the constrictor

muscles of the pharynx serve to prevent the regurgitation of blood from the gut to the pharynx.

Relaxation of the longitudinal extensor muscles of the epipharynx followed by a contraction of the extensors of the pre-pharynx lifts the epipharynx upward against the labrum, clearing the oral channel. The blood is drawn inward and the first phase of the feeding process begins again.

Except for the functions described for the longitudinal extensor muscles, the action of the mouth parts as given by Hughes complements the above interpretation. In describing the action of the chelicerae he stated that—"the protracted chelicerae with their flattened mesial surfaces and ventro-lateral flanges, constitute a tube opening anteriorly below the chelae and postventrally within the rostrum (beak). At the posterior end of the flanges, where they are less pronounced, the chitin is bevelled off so that it fits the dorsal ridge of the epipharynx, the ventral flange (rod) of which is held in the hypostomal groove, and the end of the rostrum (beak) is closely applied to the outside of the cheliceral shafts. Thus, the protracted chelicerae in effect constitute a tubular extension through the preoral canal, and blood will be sucked up the cheliceral tube and passing round the edges of the epipharynx will flow along the preoral canal in its posterior region ventral to the paralabra externa, which, extended laterally, close this gutter dorsally."

Hughes regarded the labrum as being twofold in function.

"First, as the chelicerae slide forward, their bases, or at any rate the basal part of the penultimate sections, come to lie above it and to press on it. This depresses the labrum so that the grooved ventral surface fits over the shallow ridge (fin-like extension) on the posterior part of the dorsal epipharyngeal wall. This downward pressure of the labrum will help to depress the epipharynx . . . . and also help to hold the epipharynx in a median position which is essential for the function of the whole complex as a telescopic tube. Secondly, as the chelicerae are withdrawn . . . . the tip of the labrum becomes inserted into the ventral opening of the cheliceral tube as it slides back. The effect of this is to part the chelicerae and to scour any blood retained between them. This function is a necessary one, since blood in the cheliceral tube is unmixed with saliva. The styles end near the tip of the rostrum (beak), and there is no evidence to indicate that blood mixes with saliva before entering rostral part of feeding canal. Blood retained by the chelicerae on their withdrawal is therefore still coagulated. The blood forced out of the cheliceral tube, as it is withdrawn and as the two halves separate as they pass the labrum, will run ventrally round the edges of the epipharynx into the hypostomal gutter, and so can become sucked back with saliva into the pharynx. By this means coagulated blood is prevented from being carried back in with the chelicerae as they are retracted."

DISCUSSION

Snodgrass (1948) has summarized the morphological information available in the older literature concerning the mouth parts of the ACARINA. In the present study an attempt has been made to relate the morphology of the mouth parts with the musculature and on this basis to postulate the probable feeding mechanism. Comparison with studies on ticks has been useful in this connection as well as in connection with an attempt to determine the probable homology of various parts. Hughes (1949) in his study on the mouth parts of *B. bacoti* included an historical survey concerning the possible homology of parts as described by other authors. His discussion on the relationships of the mouth parts of *B. bacoti* with those found in ticks is quite inclusive and will not be considered in the present paper.

Concerning the structures as found in *B. bacoti,* the following discrepancies are to be noted between Hughes' paper and that of the writer:

The interpretation of the term epistome, called the subcheliceral plate by Hughes, is based on the definition as given by Snodgrass (1948), *i.e.*, a structure "usually united with the pedipalp coxae, forming a bridge between their dorsal surfaces, always giving origin either directly or by means of a basal apodeme, to the dorsal dilator muscles of the pharynx."

The term epistome as used by Hughes here refers to the tectum or that structure which "forms the dorsal wall or roof of the capitulum," (Snodgrass, 1948).

The name capitular groove is introduced to designate the medial ventral groove of the basis capituli, a possible modification of the deutosternum, which Hughes called the hypostome. Hypostome is here reserved for that portion of the beak which lines the floor of the oral cavity.

Hughes used rostrum to refer to the entire arrangement of mouth parts supported by the basis capituli. This has been replaced by the term beak since rostrum usually refers to a dorsal projection over the mouth parts.

Explanations of discrepancies between Hughes' study and that presented here are incorporated in the body of the paper under the structures concerned. Hughes did not indicate whether his study is exclusively derived from a study of adult mites. This may account for some of the noted discrepancies since the present study deals only with adult female mites. For example, Hughes described the presence of a hood over the chelae. During the present study, a covering was observed in the nymphal stages but was never found in the adult female mite. It is possible that Hughes has presented a composite picture of the structure of the gnathosoma of *B. bacoti* derived from adults and nymphs.

SUMMARY

The detailed anatomy of the mouth parts of *Bdellonyssus bacoti* has been studied through the use of live mites, mounts of dissections showing individual parts and sectioning technics. An oral channel surrounded by the hypostome, mala interna, mala externa, epipharynx, styles, stylets and labrum passes through the beak. At the junction of the beak and the basis capituli the channel opens through a pharyngeal orifice into the pharynx. An epistome separates the pharyngeal chamber from the cheliceral region and performs a number of mechanical functions. Observations on feeding mites indicate that mites will feed on broken or intact skin; the time of engorgement varies from 6 to 17 minutes. A postulated mechanism of feeding is proposed from a study of the structures and musculature involved. The chelicerae pierce the skin and cause a pool of blood to form. Blood is drawn through the oral channel into the pharynx by the action of hypostomal and pharyngeal muscles. By the action of certain extensor muscles the pharyngeal orifice is then closed by the epipharynx to prevent regurgitation and the blood propelled into the gut by waves of contraction of pharyngeal muscles. At the posterior end of the pharynx dilator muscles are absent and the constriction caused by the constrictor muscles serves to prevent regurgitation from the gut.

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Symbols

A.—dorsal pair of longitudinal extensors
B.—ventral pair of longitudinal extensors
B.B.—base of bifid process (tritosternum)
B-L.P.—blade-like processes (lacinae) of bifid process
B.Max.—base of styles
B.P.—bifid process (tristosternum)
C.—lateral pair of longitudinal extensors
C.A.—capitular attachment—camerostome
C.M.Ph.—ventro-lateral constrictor muscles of pharynx
C.P.—central portion (paralabrum internum) of labrum
C.S.Ct.G.—convex sides of capitular groove
C.S.Li.G.—convex sides of hypostome
Chl.—shafts of chelicerae
Chl.Sh.—outer cheliceral sheaths
Chl.Sh′—inner cheliceral sheaths
Ct.G.—capitular groove
Ct.T.—capitular teeth
I-Cxp.—mala externa
II-Cxp.—mala interna
D.—dorsal constrictor muscle of pharynx
D.L.D.—dorso-lateral dilators of pharynx
D.Ct.—dorsal surface of capitulum
D.Ph.—dorsal surface of pharynx
D.M.Hst.—dilator muscles of hypostome
D.M.Ph.—dilator muscles of pharynx
E.L.—longitudinal extensors of epipharynx
E.P-ph.—longitudinal extensors of pre-pharynx
E.S.E.P.—ventral extensions of epistome
F.—sclerotic flap of trochanter (second palpal segment)
F.E.—fin-like extension of dorsal edge of epipharynx
F.H.—forked hair on tarsus (sixth palpal segment)
F.U.C.S.—forked union of convex sides of hypostome
Fl.—flange of chelicerae
Hst.—hypostome
Hst.T.—hypostomal teeth
L.—epipharynx
L.A.—aperture of epipharynx
L.B.—base of epipharynx
L.D.—lateral digit of chelicerae (movable digit)
L.E.—longitudinal extensors
L.E.Ph.—longitudinal extensors of pharynx
L.R.—rod on ventral edge of epipharynx
L.T.—teeth of epipharynx
Li.—floor of oral channel
Li.G.—floor of hypostome
M.—membrane of tritosternum
M.D.—medial (immovable) digit of chelicerae
M.E.—paralabrum externum of labrum
Max.—style

SYMBOLS

Max.G.—groove of style
O.C.—oral channel
Oes.—oesophagus
P.—palp
Ph.—pharynx
P-ph.C.—pre-pharyngeal chamber
Ph.O.—pharyngeal orifice
R.—rod of mala externa
R.P-ph.—region of pre-pharynx
S.—setae
S.E.P.—epistome
S.P.—stipes of palpi
Slv.—salivary glands
Sp.—spiracle on sternal plate
Sph.—constrictors of pharynx
St.P.—sternal plate
S.I-Cxp.—stylus of mala externa
T.L.—teeth of epipharynx
T.P.Li.—triangular plates of hypostome
Tect.—tectum capituli
V.—labrum
V.B.—base of labrum
V.L.—ventro-lateral constrictor muscles of pharynx
V.L.D.—ventro-lateral dilators of pharynx
V.L.Ph.—ventro-lateral walls of pharynx
L.R.—rod-like thickening on ventral edge of epipharynx
V.S.E.P.—ventral surface of epistome

Explanation of Plates

Plate I

Fig. 1. Diagrammatic sagittal section of entire capitulum. Scale at top of figure shows approximate region through which transverse sections pass. The numbers represent the figure numbers on Plates III and IV.

Fig. 2. Ventral aspect of capitulum.

Fig. 3. Whole mount of detached tritosternum and sternal plate, ventral view.

Plate II

Scale drawings of whole mounts of individual structures of the beak removed from different mites.

Fig. 4. Ventral view of hypostome, pressed to expose mala interna and mala externa. Hypostomal groove mechanically split.

Fig. 5. Ventral view of half of hypostome and intact hypostomal groove.

Fig. 6. Ventral view of epipharynx.

Fig. 7. Hypostome manipulated to expose hypostomal teeth.

Fig. 8. Ventral view of labrum.

Fig. 9. Ventral view of entire right and portion of left chelicerae.

Fig. 10. Dorsal view of right palp.

Fig. 11. Stylet lateral to labrum.

Fig. 12. Dorsal view of right style.

Plate III

Selected transverse sections (10 micra) of capitulum arranged in serial order. Figs. 13–18 and 23 were drawn by camera lucida from a single mite; fig. 19 was drawn by camera lucida from a second mite; figs. 20–22 and 24 were drawn from a third mite at a slightly different scale.

Fig. 13. Through distal end of beak, showing interlocking structures forming the oral opening.

Fig. 14. Through beak, immediately anterior to hypostome, showing thickened mala interna and mala externa.

Fig. 15. Through beak at distal end of hypostome.

Fig. 16. Through beak at level of fin-like expansion of epipharynx. Also through articulation of cheliceral digits.

Fig. 17. Through beak at distal end of labrum.

Fig. 18. Through beak, anterior to articulation of epipharynx and bases of styles.

Fig. 19. Through beak at level of articulation of lateral edges of epipharynx with toothed ridge of bases of styles.

Fig. 20. Through beak where dorsal edges of mala interna touch bases of styles.

Fig. 21. Near junction of beak and basis capituli at level of base of epipharynx and posterior end of hypostome.

Fig. 22. At junction of beak and basis capituli and posterior end of tectum, showing fusion of dorso-lateral edges of pre-pharynx with extensions of epistome.

Fig. 23. Through basis capituli, posterior to junction with beak, showing fusion of ventral edge of pre-pharynx with capitular groove and ventro-lateral dilator muscles of pre-pharynx.

Fig. 24. Through basis capituli at level of aperture of epipharynx showing longitudinal extensor muscles entering epipharynx.

Plate IV

Continuation of transverse sections through basis capituli. Figs. 25 and 28–34 are from the first mite of Plate III; figs. 26 and 27 are from the third mite.

Fig. 25. Through posterior end of pre-pharynx, showing absence of dorsal constrictor and dilator muscles.

Fig. 26. Through anterior end of pharynx, showing first appearance of third pair of longitudinal extensor muscles.

Fig. 27. Immediately posterior to previous figure, showing separate origins of dorsal dilator muscles.

Figs. 28 and 29. Through neck of tritosternum.

Figs. 30 and 31. Through bifurcated region of epistome.

Figs. 32 and 33. Through origin of longitudinal extensor muscles.

Fig. 34. Through constrictor muscles of pharynx at level of camerostome.

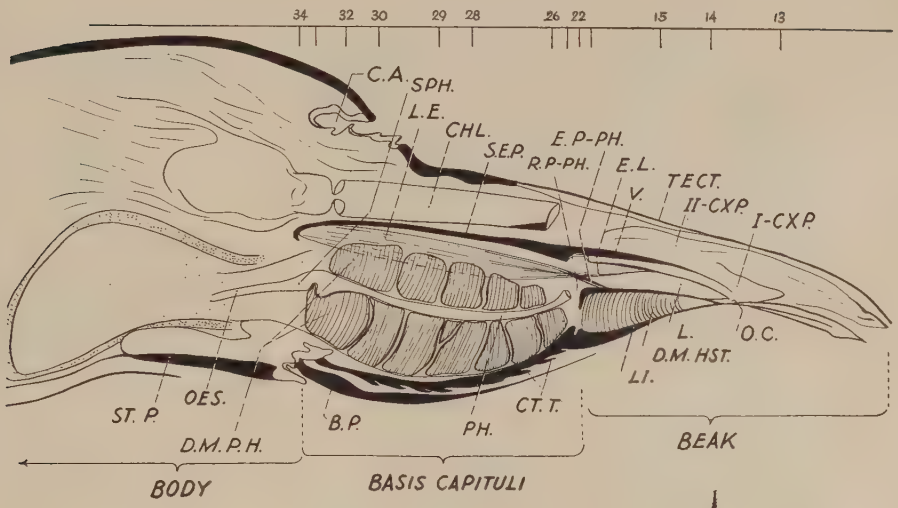


Fig. 1

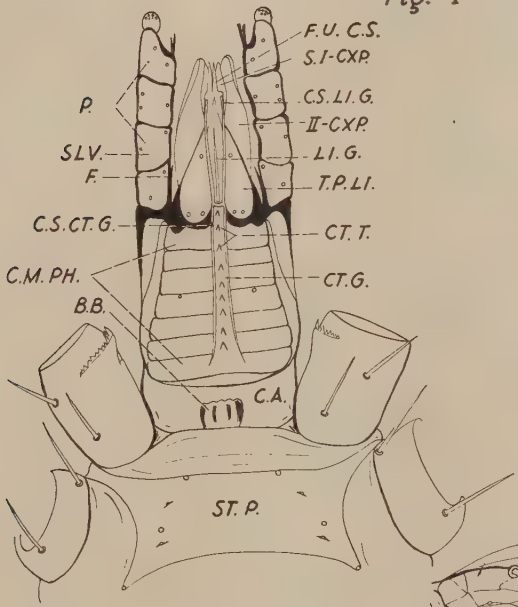


Fig. 2

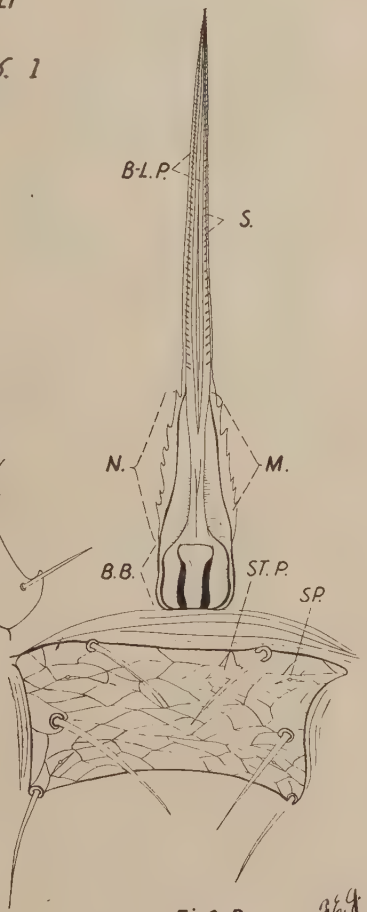


Fig. 3

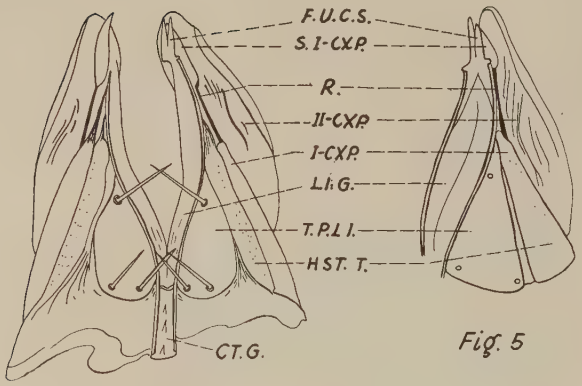
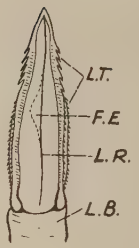


Fig. 4

Fig. 5



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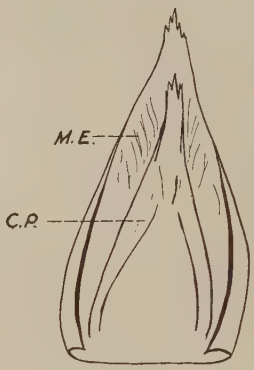


Fig. 8

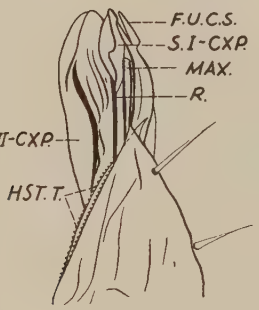


Fig. 7

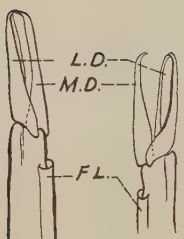
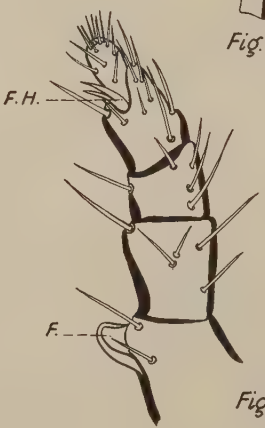


Fig. 9

100 μ

Fig. 11



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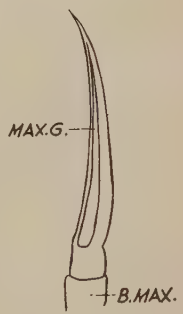


Fig. 12

JEJ 49

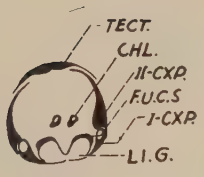


Fig. 13

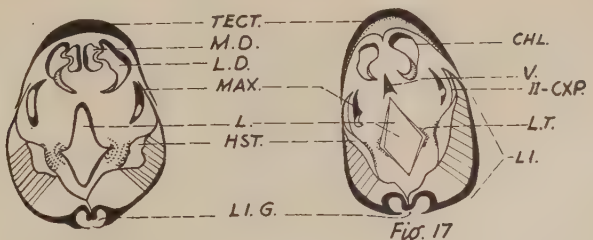


Fig. 16 Fig. 17

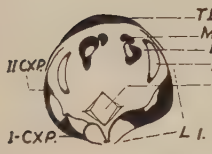


Fig. 14

50 μ

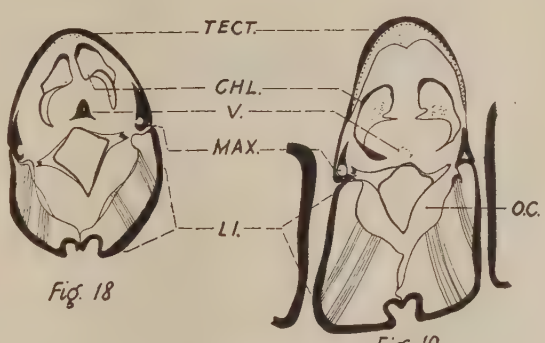


Fig. 18 Fig. 19

Fig. 15

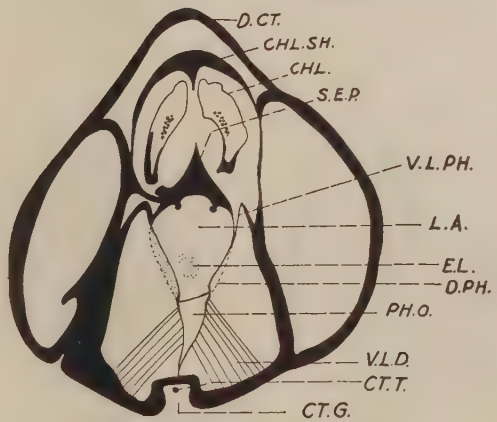


Fig. 23

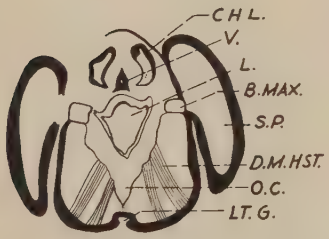


Fig. 20

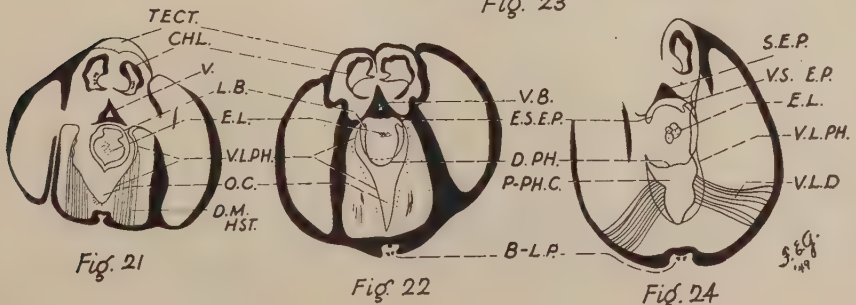
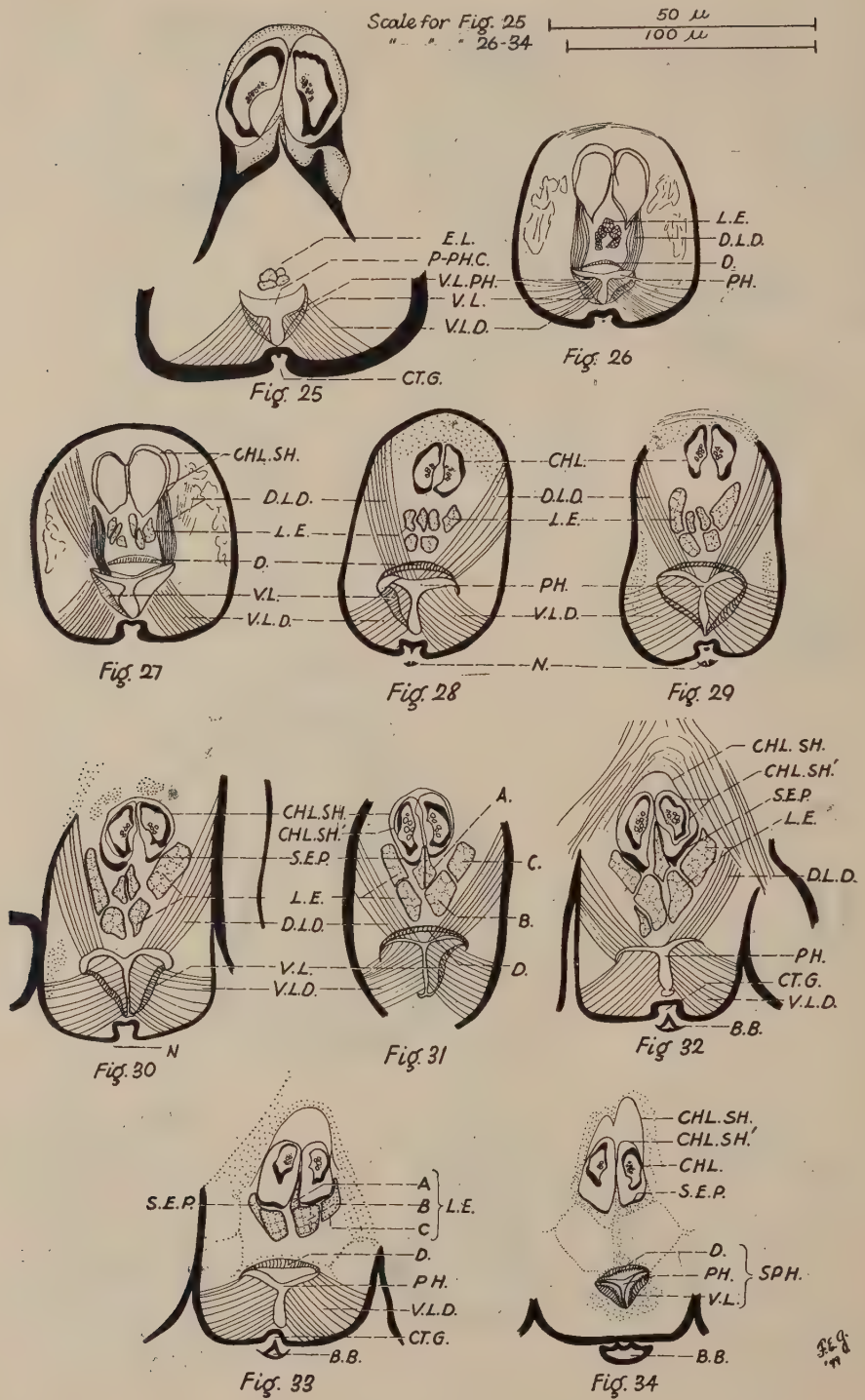


Fig. 21 Fig. 22 Fig. 24


Scale for Fig. 25 50 μ
" " " 26-34 100 μ
E.L.
P-PH.C.
V.L.PH.
V.L.
V.L.D.
CT.G.
Fig. 25
L.E.
D.L.D.
D.
PH.
Fig. 26
CHL.SH.
D.L.D.
L.E.
D.
V.L.
V.L.D.
Fig. 27
CHL.
D.L.D.
L.E.
PH.
V.L.D.
N.
Fig. 28
Fig. 29
CHL.SH.
CHL.SH.'
S.E.P.
L.E.
D.L.D.
V.L.
V.L.D.
N
Fig. 30
A.
C.
B.
D.
Fig. 31
CHL. SH.
CHL.SH.'
S.E.P.
L.E.
D.L.D.
PH.
CT.G.
V.L.D.
B.B.
Fig. 32
S.E.P.
A
B
C
L.E.
D.
PH.
V.L.D.
CT.G.
B.B.
Fig. 33
CHL.SH.
CHL.SH.'
CHL.
S.E.P.
D.
PH.
V.L.
SPH.
B.B.
Fig. 34

TWO NEW SPECIES OF TICKS FROM CEYLON (ACARINA: IXODIDAE)[1]

GLEN M. KOHLS

United States Public Health Service

The two new species described below were found among 28 lots of ticks collected from various hosts in Ceylon by W. W. A. Phillips and sent to the writer for identification by Gordon B. Thompson, Cambridge, England.

Ixodes ceylonensis new species.

Female: Color of capitulum, scutum, and legs, reddish brown.

Capitulum. Length, tips of palpi to posterior margin of basis capituli, 0.96[2]; width of basis 0.57. Cornua short and broadly rounded, the posterior margin of the basis between them nearly

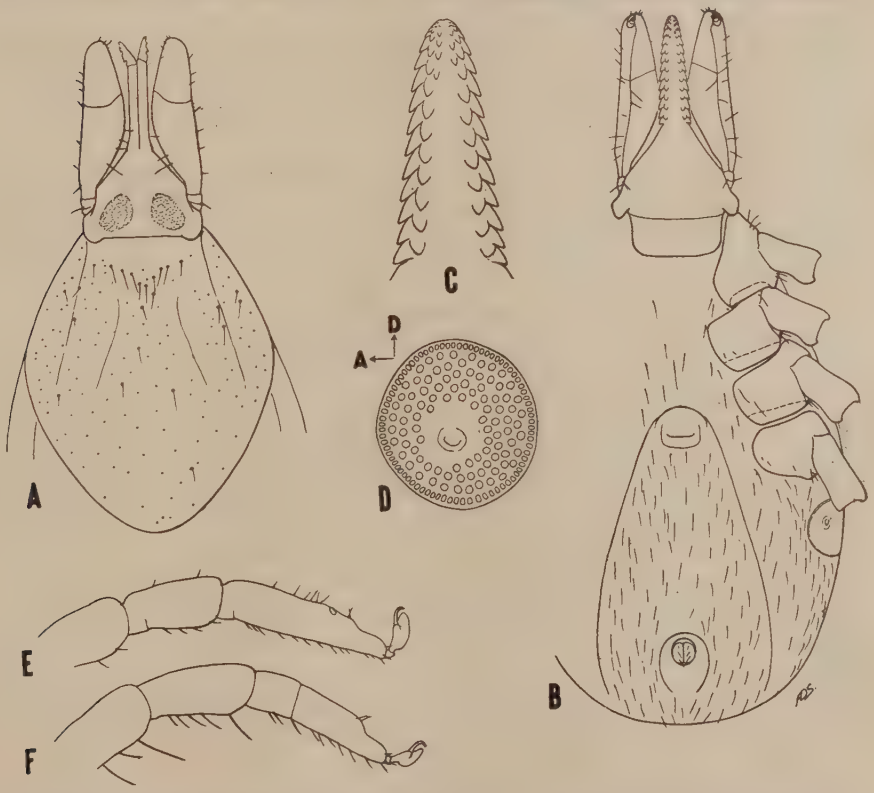


FIG. 1. *Ixodes ceylonensis* new species. Female. A. Capitulum and scutum, dorsum. B. Capitulum and body, venter. C. Hypostome. D. Spiracular plate. E. Tarsus and metatarsus, leg I. F. Tarsus and metatarsus, leg IV.

Received for publication, February 20, 1950.

[1] Contribution from the Rocky Mountain Laboratory (Hamilton, Montana), National Institutes of Health.

[2] All measurements in millimeters.

straight. Surface of basis crazed, porose areas large, ovoid, depressed, the interval between them less than the shortest axis of one. Palpi slender, outer border nearly straight, inner border convex, distal half of article 2 with a dorsal depression which contours the inner margin of the article. Combined length of articles 2 and 3, 0.75. Ventrally the basis is broad and flattened, and narrowed behind the stout, posteriorly directed auriculae; posterior margin broadly rounded. Transverse sutural line present. Palpi flattened on their inner faces. Article 1 without anterior or posterior spurs.

Hypostome. Length about 0.52. Tapered from base to the rounded apex. Denticles arranged 3/3 near the tip, then 2/2 to the base. Lateral denticles much the larger.

Scutum. Length, 1.39; width, 1.09; broadest at about the middle. Emargination shallow. Lateral carinae distinct and nearly straight. Cervical grooves shallow, divergent and terminating before reaching the posterolateral margins of the scutum. Punctations distinct, scattered; smaller ones rather numerous; larger ones fewer in number, each with a hair. Surface glossy.

Legs. Coxae flattened; I and IV each with a very small external spur; I, II, and III with salient posterior edges. Tarsi rather abruptly narrowed subapically. Length of tarsus I, 0.70; metatarsus, 0.37. Length of tarsus IV, 0.57; metatarsus, 0.44.

Spiracular plate. Nearly circular, greatest diameter about 0.36.

Genital aperture. Situated at the level of the interval between coxae III and IV.

Anal groove. Short, horse-shoe shaped.

Male and immature stages unknown.

Holotype. Female, from the mongoose, *Herpestes smithii zeylanicus* Thomas, Namunukula, Galapitakande, Uva Province, Ceylon, October 6, 1946, Rocky Mountain Laboratory No. 26890. Deposited in the Rocky Mountain Laboratory, Hamilton, Montana.

Paratype. Female, from *Rattus rattus kandianus* Kelaart, Namunukula, Uva Province, Ceylon, August 20, 1947, Rocky Mountain Laboratory No. 27080. Deposited in the British Museum (Natural History), London, England.

In the characters of the hypostome, auriculae, legs, spiracular plate, and anal groove, the new species strongly resembles *Ixodes radfordi* Kohls 1945, a species as yet known only from *Rattus rattus rufescens* in Manipur State, eastern India. It is readily distinguished from *I. radfordi* however by its larger size and by characters of the basis capituli and of the scutum. The basis is broader in the new species, cornua are present and lateral carinae are absent. The scutum is shaped differently in *I. ceylonensis* and the punctations are fewer and less conspicuous.

Haemaphysalis minuta new species

Male: Very small, length from tips of palpi to posterior margin of body, from 1.27 to 1.57 (6 specimens); width from 0.88 to 1.02. Shape ovoid, widest ahead of the spiracles.

Capitulum. Length from tips of palpi to tips of cornua about 0.31; width of basis, 0.22 to 0.25. Lateral margins of basis mildly convergent posteriorly; posterior margin between the cornua nearly straight. Surface of basis with a few scattered punctations. Cornua moderate, pointed. Palpal article 2 strongly salient laterally and longer than article 3. Article 3 without dorsal spur. In ventral view the surface of the basis is flattened and the posterior margin nearly straight. Palpal article 2 with a blunt retrograde spur at the ventrolateral angle and the inner margin with 6 or 7 flattened hairs directed toward the base of the hypostome. Article 3 with a very short, pointed retrograde spur.

Hypostome. Shape as figured. Corona moderate, dentition 4/4 with 7 or 8 denticles in each file. Principal denticles in each file about equal in size. Length about 0.175.

Scutum. Length from tips of scapulae to posterior margin ranges from 1.09 to 1.33. Lateral grooves well defined and enclosing one festoon on each side. Cervical grooves short and inconspicuous. Punctations rather coarse and deep, and evenly distributed. Festoons longer than broad.

Legs. A moderate spur on coxa I, spurs progressively shorter on II, III, and IV. All trochanters with short, pointed ventral spurs which diminish in size from I to IV. Length of tarsus, I, 0.36; metatarsus, 0.21. Length of tarsus IV, 0.30; metatarsus, 0.19.

Spiracular plate. Shape as figured. Greatest dimension about 0.19.

Genital aperture. Situated between coxae II.

Female and immature stages unknown.

Holotype. Male, from the junglefowl, *Gallus lafayettii* Lesson, Nikawewa Camp, near Kantalai, Eastern Province, Ceylon, December 31, 1938. Rocky Mountain Laboratory No. 26883. Deposited in the Rocky Mountain Laboratory, Hamilton, Montana.

Paratypes. Two males with data as for the holotype. Three males from *Gallus lafayettii,* Veddikachchai Intermediate Zone, Godapatagalla, North Central Province, Ceylon, December 30, 1940, Rocky Mountain Laboratory No. 26882. Deposited in the British Museum (Natural History), London, England, and in the collection of Gordon B. Thompson, Cambridge, England.

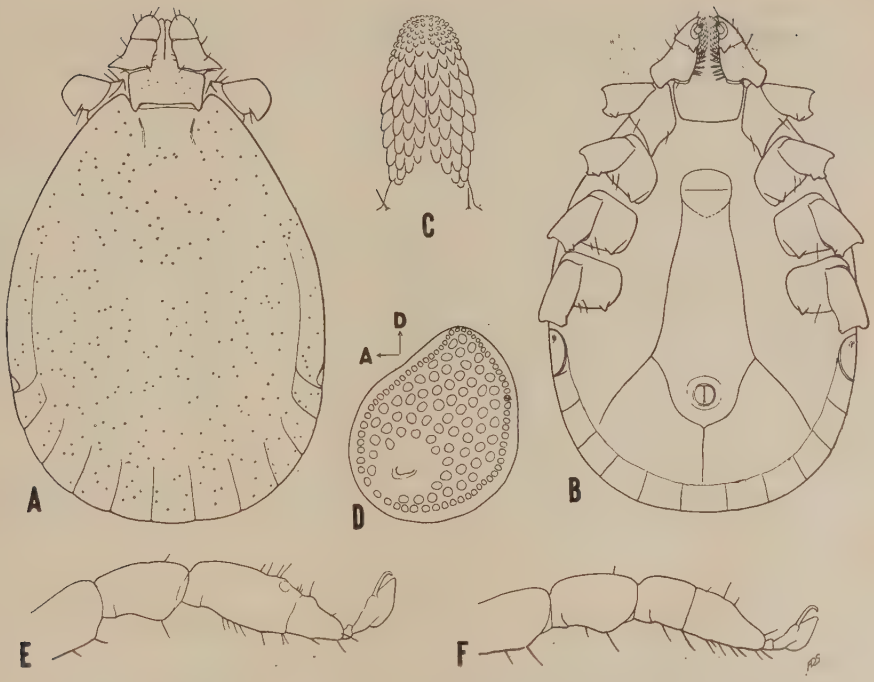


FIG. 2. *Haemaphysalis minuta* new species. Male. A. Capitulum and scutum, dorsum. B. Capitulum and body, venter. C. Hypostome. D. Spiracular plate. E. Tarsus and metatarsus, leg I. F. Tarsus and metatarsus, leg IV.

This very small species resembles *Haemaphysalis doenitzi* Warburton and Nuttall, 1909, which to the present has been known only from four males and three females found on a water hen, St. John's Island, Singapore. The writer has recently received two lots of *H. doenitzi* collected in Selangor, Malaya, by Dr. C. B. Philip and Major R. Traub. One consisted of a single male from an undetermined quail at Sungei Way, May 31, 1948; the other, two females from *Centropus bengalensis javanensis* at Kuala Lumpur, April 20, 1940. Comparison of *H. minuta* with the *H. doenitzi* male from Malaya and with the descriptions and figures of the latter (Warburton and Nuttall, 1909; Nuttall and Warburton, 1915) reveals that *H. minuta* differs in several respects including the presence of the retrograde spur on palpal article 2 ventrally, shorter lateral grooves which cross one instead of two festoons on each side, the longer spurs on coxae I, and the spurs on the trochanters ventrally.

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LABORATORY STUDIES OF COMBINATIONS OF PIPERONYL CYCLONENE, PIPERONYL BUTOXIDE, PYRETHRINS, AND ROTENONE FOR THE CONTROL OF TICKS ON DOGS

DONALD MOORE

U. S. Industrial Chemicals, Inc., Research and Development Laboratories, Baltimore, Maryland

It has been reported by Wachs (1947) that synergism could be demonstrated when piperonyl butoxide or piperonyl cyclonene was used in combination with pyrethrins for the control of houseflies. Brannon (1947) likewise demonstrated the principle of synergism when either piperonyl butoxide or piperonyl cyclonene was used in combination with rotenone for the control of the Mexican bean beetle. When Dove (1947) reported on the relative safety of piperonyl butoxide to warm-blooded animals, it was felt that investigations should be made on similar combinations to determine the relative effectiveness against dog ticks.

The present paper summarizes the results of several laboratory tests with these combinations for the control of the American dog tick, *Dermacentor variabilis* Say and the brown dog tick, *Rhipicephalus sanguineus* Latr.

MATERIALS

Basic preparations of the emulsions and dusts and the concentrations and combinations in which they were used are listed as follows:

1. T-195 Emulsifiable Concentrate
   (10 grams piperonyl butoxide with 0.5 grams pyrethrins per 100 ml.) at 1–100 and 1–50
2. T-328 Emulsifiable Concentrate
   (4 grams piperonyl butoxide with 1 gram rotenone) at 1–50 and 1–25
3. Emulsifiable Concentrate
   (8 grams piperonyl butoxide with 0.2 grams pyrethrins and 1.0 grams rotenone per 100 ml.) at 1–200, 1–100, and 1–50
4. Dust
   0.625 per cent piperonyl cyclonene with 0.05 per cent pyrethrins in pyrophyllite carrier
5. Dust
   0.5 per cent piperonyl cyclonene with 0.05 per cent pyrethrins and 0.25 per cent rotenone in pyrophyllite carrier
6. Dust
   1.0 per cent piperonyl cyclonene, 0.1 per cent pyrethrins with 0.5 per cent rotenone in pyrophyllite carrier
7. Dust
   0.3125 per cent piperonyl cyclonene with 0.025 per cent pyrethrins in pyrophyllite carrier

Received for publication, April 3, 1950.

METHODS

Emulsions and powders were tested against both species of ticks that commonly occur on dogs in the United States. The emulsions were made of emulsifiable Pyrenone[1] concentrates diluted with water to the desired concentration of the active ingredients. Fifteen gallons of dilute insecticide were put into a 25-gallon tank, in which the dogs were completely immersed. The ticks were counted when thorough examinations were made of the test animal immediately before the dipping and again at 24 and 72 hours after the dipping. The number of ticks varied in the different tests from 10 to 137. Infestations with the American dog tick were accomplished by artificially infesting each dog with 50 adult ticks and allowing them to become attached prior to treatment. Infestations of the brown dog tick were obtained by placing the dog in a pen known to be heavily infested with brown dog ticks. Sufficient time was allowed to elapse for the ticks to become engorged before the animal was treated. In these tests different nymphal and adult stages of ticks were represented.

In treating the animals, one ounce of dust was applied from a shaker can to each average-size animal, taking care to work it into the hair and for thoroughly covering the entire animal. As in the emulsion tests, ticks were counted before treatment, also at 24 hours after the treatment.

RESULTS

The results of the tests are summarized in Tables 1 and 2. While the infesta-

TABLE 1.—*Effectiveness of Emulsions Containing Piperonyl Butoxide with Pyrethrins or Rotenone, or Both, Used in Dips for Dogs Infested with the American and the Brown Dog Ticks. Baltimore, Maryland, 1949.*

Kind of Tick on Dog	Formula Number*	Concentration of Dip Used in Grams per 100 ml. of Dip			Number of Ticks Present	Per Cent Mortality—Hrs.		
		Butoxide	Pyrethrins	Rotenone		24	72	96
American	1	0.2	0.01	...	26	96	..	96
American	1	0.2	0.01	...	18	100	..	..
Brown	1	0.1	0.005	...	67	83	..	97
Brown	1	0.1	0.005	...	113	68	..	85
Brown	1	0.2	0.01	...	65	85	..	91
Brown	3	0.16	0.004	0.02	17	24	..	..
Brown	3	0.08	0.002	0.01	12	17	..	..
Brown	3	0.04	0.001	0.005	11	0	..	..
Brown	2	0.08	....	0.02	10	..	50	..
Brown	2	0.16	....	0.04	137	..	90	..

* See text.

TABLE 2.—*Effectiveness of Dusts Containing Combinations of Piperonyl Cyclonene, Pyrethrins, and Rotenone Against the American Dog Tick and the Brown Dog Tick, Baltimore, Maryland, 1949.*

Kind of Tick on Dog	Formula Number*	Concentration of Dust Used in Per Cent			Number of Ticks Present	Per Cent Mortality 24 Hrs.
		Cyclonene	Pyrethrins	Rotenone		
American	4	0.625	0.05	...	23	22
American	5	0.5	0.05	0.25	6	50
American	6	1.0	0.1	0.5	30	94
Brown	4	0.625	0.05	..	101	99
Brown	7	0.3125	0.025	..	116	89

* See text.

[1] U. S. Industrial Chemicals, Inc., registered trademark indicating a combination of piperonyl butoxide with pyrethrins.

tions varied considerably in the various test animals, definite trends could be noted. An emulsion concentrate that appeared to be highly effective against both species of ticks was one containing 0.2 grams of piperonyl butoxide and 0.01 grams pyrethrins per 100 ml. of dip. It will be noted in Table 1 that 0.1 per cent piperonyl butoxide and 0.005 per cent pyrethrins also gave good control of brown dog ticks. A spray containing 0.16 per cent piperonyl butoxide and 0.04 per cent rotenone was also effective against brown dog ticks.

Both dipping and dusting tests were conducted on dogs infested with the American dog tick and the brown dog tick. Only adults of the American dog tick were used, while all stages of the brown dog tick were employed. Various concentrations of combinations of piperonyl cyclonene or piperonyl butoxide with pyrethrins and rotenone were tested in dusts and emulsions. A dip containing 0.2 grams of piperonyl butoxide and 0.01 grams of pyrethrins could be recommended as a control of both the American dog tick and the brown dog tick. One ounce of dust containing 1 per cent of piperonyl cyclonene, 0.1 per cent of pyrethrins, and 0.5 per cent rotenone could be used to control the American dog tick, and a dust containing 0.625 per cent cyclonene and 0.05 per cent pyrethrins could be used to control the brown dog tick. Weekly applications of either the dust or emulsion throughout the tick season would readily control both types of ticks. No ill effects were seen in any of the treated animals, which supports an earlier report (Sarles, 1949) of the nontoxic effect of this material on warm-blooded animals and man.

These findings show that an emulsion concentrate containing 10 per cent piperonyl butoxide and 0.5 per cent pyrethrins diluted with 49 equal volumes of water can be used effectively as a spray or as a dip for control of both species of dog ticks.

A concentrate containing 10 per cent piperonyl butoxide and 1.0 per cent pyrethrins, which is generally available throughout the United States for control of pests about foods, feeds, and upon animals, also has been found suitable for control of both species of dog ticks when used at a dilution of 1 to 100. It has also had extensive use as a surface spray in kennels at a dilution of 1 to 10, with a high degree of practical control from a single application. It is applied to the hiding places of the ticks that have dropped from the dogs. Its rather general use for control of different insect pests about the farm favors its use for dog ticks by the individual farmer.

In a program for control of ticks on all dogs in a community, the concentrate containing 10 per cent piperonyl butoxide and 0.5 per cent pyrethrins could be used effectively and economically. Such a dip could be mixed in a container and transported by truck from house to house, and dogs be quickly dipped. A warm day should be chosen to avoid chilling of the animals.

SUMMARY

1. Some safe and effective treatments have been developed for control of brown dog ticks *Rhipicephalus sanguineus* and American dog ticks *Dermacentor variabilis* on dogs. Either dips, sprays, or dusts are applicable for control of such pests by community effort.

2. The same dusts or spray concentrates are also effective for the treatment of buildings that are infested with these two commonly found species of dog ticks.

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OBSERVATIONS ON ECTOPARASITES OF SOME SMALL MAMMALS IN EVERGLADES NATIONAL PARK AND HILLSBOROUGH COUNTY, FLORIDA[1]

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INTRODUCTION

During 1948 and 1949 numerous small mammals were collected in Florida in connection with studies on murine typhus fever. This paper contains data pertaining to the ectoparasites of some of these animals. A contrast between findings in Hillsborough County and in Everglades National Park is presented.[2]

MATERIALS AND METHODS

Trapping in the Everglades took place in the latter half of January, 1949, and was confined to the region within a few miles' radius of Park Ranger Station No. 1, about 12 miles southwest of Homestead, Florida. This station is situated in a hammock. Such hammocks, which may measure a half mile in diameter, are densely wooded and slightly elevated above the surrounding plains. The region has been described by Davis (1943).

Where the land is somewhat less elevated, a region of pine trees occurs, while at or near ground water level grasslands stretch for many miles. An unimproved road leading west of the Ranger Station passes through about six miles of pine woods, interspersed with hardwood hammocks in high places and extensive truck gardens in low ones. The road continues two miles beyond the pines through increasingly moist grasslands that were once cultivated, and finally ends in the midst of a vast marshy plain. The road is paralleled by a drainage ditch cut vertically through the underlying limestone (Figure 1).

Most of the animals were trapped along the latter two-mile stretch of road, while the remaining ones were caught in hammocks. None was trapped in the pine regions, apparently due to lack of ground cover.

Trapping in Hillsborough County took place chiefly in the regions immediately north and northwest of Tampa. The animals given detailed consideration in this paper were obtained in late February and early March, 1949, so that only about a month's interval separates the two collections. Physical environmental factors presumably play a more important role in the contrasts observed than seasonal differences. The Hillsborough region is in general much drier than the Everglades; it receives less rainfall, is higher in elevation, and has a sandy soil that lends itself to rapid drainage. Trapping was conducted along roadsides and on a variety of farms, chiefly citrus groves and cattle ranges. The habitats did not vary much among

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FIG. 1. Everglades road along which most of the trapping was done. The observer is looking east toward the pine regions.

FIG. 2. Abandoned field in Hillsborough County. Cotton rats abundant in field; cotton rats and rice rats both present in cypress region in background.

themselves, since an attempt was made to select trapping stations along fence-rows or other boundaries where grass and low shrubbery grew profusely, and, when possible, such stations were chosen near streams or other small collections of water. All these situations were subject to changes that commonly occur in populated rural districts, thus differing markedly from the isolated aspect of the Everglades study area.

Trapping in the Everglades was conducted for 15 consecutive nights. Four types of traps were used: small metal Sherman traps; wooden box traps, Richter and Emlen (1945); and small and large wire-mesh "Hav-a-hart" traps. Stations were set at 0.2-mile intervals along the western end of the road beyond the pine regions. Elsewhere they were placed at the edge of, or within, hardwood hammocks.

Traps were visited as early as possible each morning. The animals were transported at once to field headquarters in individual compartments of a carrying cage. Exchange of ectoparasites during transit was unlikely, as the compartments were separated by wooden partitions. Ectoparasites rarely showed themselves before the animals were actually handled.

At headquarters the animals were driven into separate cloth sacks and given nembutal anesthesia by intraperitoneal injection. Combing was then performed in bright light into a white enamel pan. All ectoparasites were placed in vials of 70 per cent alcohol.

Even vigorous combing did not result in the recovery of all ectoparasites, for subsequent examination through a 5-x lens revealed a variable number of residual ticks, lice, parasitoid mites and trombiculid mites. As many as possible of such remaining parasites were picked off by hand. Thus the final census for each animal was as close to complete as possible, and comparisons of degrees of infestation are valid in so far as the method for processing the mammals was uniform.

At the Tampa laboratory the ectoparasites from each animal were sorted into their respective taxonomic groups. Fleas were identified with available keys, Ewing and Fox (1943) and Hubbard (1947), and other ectoparasites were sent to various authorities for identification.[3]

Similar techniques were employed in the case of mammals and ectoparasites from Hillsborough County.

RESULTS

In the Everglades 146 mammals were trapped, comprising 78 cotton rats, *Sigmodon hispidus spadicipygus* Bangs, 40 rice rats, *Oryzomys palustris coloratus* Bangs, 24 cotton mice, *Peromyscus gossypinus palmarius* Bangs, 3 roof rats, *Rattus rattus* (Linnaeus) subspecies, and 1 opossum, *Didelphis virginiana pigra* Bangs. Identifications were derived from Hamilton (1943).

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Henry S. Fuller, Harvard University School of Public Health, for determinations of trombiculid mites.

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The animals showed definite habitat relationships. Cotton rats, although invading dry hammocks in small numbers, were more abundant in the wetter lowlands. Rice rats seemed confined to the latter situation. Cotton mice, on the other hand, were more prevalent in the hammocks. The three roof rats were caught in an abandoned shed at the juncture of a hammock and the pine belt. Although only one opossum was caught, several were seen on various roads at night; their range covers all the local habitats.

A series of 20 consecutively trapped cotton rats and rice rats from Hillsborough County was arbitrarily selected for comparison with others from the Everglades. Both these species are widely distributed within the county, although the rice rat shows a marked, but not absolute, restriction to watery habitats. These rats are of different subspecies than the Everglades specimens. Hillsborough County rice rats are designated as *Oryzomys palustris natator* Chapman, while the subspecific status of the local cotton rat is in a controversial stage, Barrington (Personal communication). It is remotely possible that some of the following contrasts in ectoparasitic populations on the different races of these host species may be due in part to physiological variations incurred during the process of morphological speciation.

Ectoparasites.—The tropical rat mite, *Liponyssus bacoti* (Hirst) (Acarina) was taken from four hosts in the Everglades, being found on 28 per cent of cotton rats, 20 per cent of rice rats, 12 per cent of cotton mice, and on all three roof rats. It was not an abundant organism; the maximum number encountered on one host, a rice rat, was 14. In Hillsborough County, during the study period it was taken from 80 per cent of cotton rats and 50 per cent of rice rats; the largest number occurring on one animal, a cotton rat, was 20. At other times it has been recovered from the following additional hosts in Hillsborough County: Norway rat, *Rattus norvegicus* (Erxleben); house mouse, *Mus musculus* (Linnaeus), marsh rabbit, *Sylvilagus palustris paludicola* (Miller and Bangs); and opossum. The cotton rat appears to be the principal host of this mite in the areas studied.

Habitat of the host appeared to have little or no influence on the distribution of *L. bacoti,* as it was recovered from all areas investigated in fairly uniform numbers. The impression was gained, however, that a moist environment was most favorable.

This mite displays a cycle of abundance during the year in Hillsborough County, becoming scarce in October and November, and building up to a maximum population in March, April and May. In the latter months trapped cotton rats were 66.7 per cent, 87.5 per cent and 100 per cent infested respectively, with maximum counts of 791, 253 and 151 mites on a single animal in the respective months.

Liponyssus bacoti was found most frequently in the deep fur of the lower back and tail-base of its rodent host. It is difficult to dislodge with the comb, as it slips in and out among the hair shafts at skin level with great dexterity. No evidence of its feeding could be detected with a 5-x lens, a finding somewhat in contrast to the known reaction to its bites in man, Bishopp (1923). The mite was readily colonized on cotton rats and rice rats in the laboratory, a finding in agreement with the work of Skaliy and Hayes (1949).

The mite, *Haemolaelaps glasgowi* (Ewing) (Acarina), was taken from 90 per cent of cotton rats, 50 per cent of rice rats and 12 per cent of cotton mice in the Everglades. The maximum infestation was 219 mites on a cotton rat. In Hillsborough County 80 per cent of cotton rats and 60 per cent of rice rats were infested, with a

maximum of 25 mites on one cotton rat. The mite has been found subsequently on roof rats and house mice. The cotton rat is the most frequent host, showing both the greatest frequency and the highest degree of infestation.

A distinctly moist environment appears to be favorable for this mite, since it was not only more prevalent in the Everglades than in Hillsborough County, but also displayed a distribution in favor of grasslands rather than hammocks.

An annual cycle for this species is harder to discern than in the case of *L. bacoti*. Presumably the rainy summer season does not depress its numbers appreciably, since it is adapted to a moist environment.

Haemolaelaps glasgowi was found on all regions of its host, although most abundantly in the groins, from where it was removed best by a sawing motion of the comb. It is rather easily dislodged and runs about actively in the pan. This mite does not become engorged, although it probably takes small blood meals. Cutaneous signs of its feeding were absent.

The recent work of Strandtmann (1949) is followed in referring this mite to the genus *Haemolaelaps* rather than *Atricholaelaps*.

A third mite, *Laelaps* sp. (Acarina), was taken only from rice rats. In the Everglades it was found on 50 per cent of these hosts, with a maximum infestation of seven mites on one rat. In Hillsborough County it was found on 10 per cent of rice rats, with a single mite as the maximum on one animal. It has also been found on a cotton rat, but the rice rat is clearly the specific host for it. This mite will be described and named by Pratt (Personal communication).

Thirty-five per cent of the rice rats caught in the Everglades harbored *Gigantolaelaps* sp. (Acarina), the most heavy infestation consisting of 10 mites. This species has not been encountered in Hillsborough County, although it is known from rice rats in southern Georgia, Morlan and Strandtmann (1949). It appears to be host-specific. Its description by Morlan and Strandtmann is forthcoming.

This large ectoparasite is adept at concealing itself in its host's pelage and resisting efforts to comb it out. As in the case of other laelaptid mites, this species was never found engorged, and visible cutaneous host-reactions to feeding could not be perceived.

Three species of chigger were recovered from animals in the Everglades. *Eutrombicula splendens* (Ewing) (Acarina), the predominant mite of this genus on both cotton and rice rats, was widely distributed in the grasslands; *Eutrombicula multisetosa* (Ewing) was found in only the wettest parts of the grasslands, with a rather marked concentration on cotton rats. *Eutrombicula batatas* (Linnaeus) was encountered once on a grassland rice rat and twice on roof rats in a hammock. This infrequent occurrence did not allow any conclusion as to its optimum host or habitat. It is known to attack man, Jenkins (1949). Only *E. splendens* was identified in Hillsborough County, being taken from 60 per cent of cotton rats and 95 per cent of rice rats during the restricted study period. As yet, it has not been recorded from additional hosts.

Counts of chigger infestations were not made, owing to the frequent occurrence of many hundreds of these mites on a single animal. It would have been difficult to decide what to include in such an enumeration, for in many cases the hosts bore many larval exoskeletons; the mites themselves had dropped off after molting. In other cases chiggers had died on the host within masses of dried serum. These

crusts sometimes constituted the sole evidence of infestation. It is interesting to note that the pinkish-red color of the larvae was retained by these various remains, so that it was possible to affirm recent chigger infestation in some animals no longer tenanted by living parasites.

In Hillsborough County *E. splendens* has been taken most frequently in the spring and summer, being especially prevalent in moist habitats.

Chiggers were found principally on the ear margins near the bases. In heavy infestations they spread radially along the margins until in a few cases the entire ear was rimmed by a conspicuous red band of ectoparasites. Chiggers were found not infrequently inside the ears, usually on the eminence of one of the folds. Occasional chiggers were seen about the base of the tail, the anus and the genital region. In cotton rats, but not in rice rats, it was not unusual to find many chiggers on the apposed surfaces of the forelegs and thorax. Here they occurred in rows corresponding to the distribution of hair follicles. The cotton rat has a rather sparse pelage in this region, while rice rats are heavily furred, which may account for the observation.

This group of mites was responsible for the most numerous cutaneous reactions resulting from ectoparasitic activity. However, sloughing of the crusts did not leave conspicuous scars.

Listrophorid mites, unidentified as to species, were observed frequently on cotton rats, rice rats and cotton mice both in the Everglades and in Hillsborough County. On some animals they occurred in enormous numbers, particularly in the hair of the flanks. An individual hair occasionally was plastered with nits from its base to its tip. No harmful effects resulting from heavy infestation were observed. When an animal was killed, the mites migrated outward along the hairs in the manner of lice on a carcass, producing a dusty appearance of the fur.

One single specimen of another mite species, *Dermatophagoides scheremetewskyi* Bogdanow (Acarina), was obtained in the Everglades from a cotton mouse trapped in a hammock. It has not been encountered in Hillsborough County.

In the Everglades *Dermacentor variabilis* (Say) (Acarina) was taken from 56 per cent of cotton rats, 47 per cent of rice rats, 94 per cent of cotton mice and all three roof rats. It was often found abundantly on these hosts, the maxima on single animals being 45, 22, 25 and 11 respectively. During approximately the same period in Hillsborough County it was taken from 40 per cent of cotton rats with a maximum infestation of 15 ticks, and from 65 per cent of rice rats with four ticks as the maximum. Subsequently the following Hillsborough County hosts have been found parasitized: roof rat; marsh rabbit; racoon, *Procyon lotor elucus* Bangs; and opossum.

At this time of the year most ticks were in the larval state, although a few nymphs were present. Adult *D. variabilis* were not acquired by the trappers. In the Everglades larval ticks were taken most frequently from cotton mice in hammocks. This may indicate a favorable host, a favorable environment, or both. There seemed to be an inverse relationship between the prevalence of ticks and mites, the former abounding in drier situations and the latter in more marshy ones.

Larval *D. variabilis* were most common on the external surface of the ear margins. Very few of them were even partially engorged although they were firmly attached. Possibly they were dormant. Nymphs situated themselves in deeper

fur of the anterior half of the host's body; most of these were in various stages of engorgement.

Scars on the outer surfaces of the ears of many of the animals indicated the most frequent sites for larval tick attachment. These did not resemble healed scars resulting from fights with other animals. Older rats and mice sometimes had so many such scars that their ear margins were rendered somewhat irregular. Single scars were circular, about one or two millimeters in diameter, and showed glistening lines of contraction proceeding from their slightly depressed centers. A scar visible on one surface of the ear did not produce a corresponding pit on the opposite side; it must therefore have been superficial.

Two adult *Ixodes scapularis* Say (Acarina) were taken from the opossum caught in the Everglades. This species has been noted in Hillsborough County on marsh rabbits. Other ticks found in the Everglades were unidentified *Ixodes* larvae recovered from cotton rats, rice rats and cotton mice. In Hillsborough County *Amblyomma maculatum* Koch (Acarina) has been found on cotton rats and roof rats, and *Haemaphysalis leporis-palustris* (Packard) (Acarina) on roof rats, marsh rabbits, and cottontail rabbits, *Sylvilagus floridanus floridanus* (Allen).

Sucking lice on cotton and rice rats exhibited host specificity in both the Everglades and Hillsborough County. *Hoplopleura hirsuta* (Ferris) (Anoplura) appeared on 12 per cent of "Everglades" cotton rats and on 10 per cent of "Hillsborough County" animals: maximum infestations were 18 and 1, respectively. Rice rats in the Everglades showed 18 per cent infestation with *Hoplopleura quadridentata* (Neumann), while 35 per cent were infested in Hillsborough County. Three was the maximum louse population on one animal in the former case, eight in the latter. *Hoplopleura oenomydis* Ferris has been taken from Norway rats in Hillsborough County.

Lice were found either lying flat on the ears in the distal zone of their outer surfaces, or in the fur of the upper back, shoulders and neck, close to the skin. If the hair at the site of louse infestation was pulled out, an area of faint cutaneous pigmentation could often be seen. This would indicate that the lice tended to inhabit constantly the areas in which they were found. Further testimony to this effect is that nits were fairly well restricted to the same regions, rather than being scattered promiscuously over the host.

Polygenis gwyni (C. Fox) (Siphonaptera) was combed from 41 per cent of cotton rats in the Everglades, with a maximum infestation of 12 fleas. Two were recovered from the opossum also, but none was present on rice rats or cotton mice. In Hillsborough County, however, the situation was quite different. One hundred per cent of cotton rats were infested, with a maximum infestation of 43 fleas, and 90 per cent of rice rats were infested with a maximum of 14 on one animal. This flea has been taken in Hillsborough County also from the Norway rat, roof rat, cotton mouse and wood rat, *Neotoma floridana floridana* (Ord). It is odd that farther north, in Georgia and North Carolina, the cotton rat is infested with a number of other wild-rodent fleats, Harkema and Kartman (1948), while during the study period in Florida it has been found carrying only *P. gwyni* and the following fleas characteristic of commensal rats or domestic animals: *Xenopsylla cheopis* (Rothschild), *Ctenocephalides canis* (Curtis), and *Echidnophaga gallinacea* (Westwood).

The cotton rat appears to be the most important host for *P. gwyni,* although the rice rat is probably a significant supplementary host in some situations.

A definite annual cycle of abundance was observed in the case of *P. gwyni.* During the fall months it reaches its lowest level, being absent from many cotton rats and only sparsely present on the others. It reaches its peak from late winter until about a month after the onset of the summer rains. Infestation rates in March, April and May, 1949, in Hillsborough County were 97.7 per cent, 87.5 per cent and 100 per cent, while maximum infestations were 39, 46 and 19 respectively.

This flea is rather large and sluggish compared with *Xenopsylla cheopis.* It adheres closely to the fur of its host and is therefore sometimes difficult to comb out. Attempts to establish it on white mice in the laboratory have been unsuccessful, owing to the ease with which the mice find and destroy them. Cutaneous reactions to the presence of *P. gwyni* were not observed in cotton rats or rice rats.

Two of the roof rats trapped in the Everglades were infested with warbles or "wolves." These maggots were of two species: *Cuterebra* sp. (Diptera) and another species somewhat resembling *Dermatobia.* The cuterebrid maggot of one of the rats was encysted in the skin of the back. The sinus was draining profusely with a dark viscid fluid that matted the hair in a long streak. The other rat had a sinus in its scrotum. Both a cuterebrid maggot and two Dermatobia-like maggots were removed from this site. Each was encysted separately, although only one sinus could be located. Since the *Cuterebra* was almost full size and the *"Dermatobia"* only half-grown, it is possible that the latter gained access to the rat's tissues through a pre-existing sinus created by the *Cuterebra.*

Warbles have been found frequently in marsh rabbits in Hillsborough County and also occasionally in roof rats and wood rats. None of these was reared successfully.

DISCUSSION

The degree of parasitization of a host is a matter of some interest, since it is usually impossible to determine why one animal harbors a large parasitic population and another animal supports only a few parasites or none at all. In some cases a visible physical deformity is the obvious cause of inability to remove vermin by natural means such as preening or scratching, Kartman (1949) and Worth (1940).

The ectoparasite records referred to in this paper were studied from the standpoint of parasite associations on single hosts, to determine whether there was a factor that could be related to degree of infestation. However, no species of ectoparasite seemed to enhance the opportunities of any other species for unusually extensive tenure. The kinds of ectoparasites on individual animals were not correlated with one another, but rather appeared to coexist with complete independence. This might have some relationship to the fact that some of the infestations showed rather sharp limitations to specific regions of the hosts' bodies. As a result there may have been only slight or no competition among the various kinds of fur inhabitants.

Random distribution of ectoparasitic species on their hosts may be surmised from the following representative data. Everglades cotton rats were infested by seven species of ectoparasites. Six of these were found on one rat, five on eight rats, four on 15 rats, three on 20 rats, two on 25 rats, and one on nine rats. These seven ectoparasite species occurred in various combinations on a host. In one case six

species were associated. There were three combinations comprising five species, nine comprising four species, six comprising three, seven comprising two, and three infestations by a single species.

It was found that total numbers of ectoparasites on cotton rats in the two regions were not significantly different, but that the kinds of ectoparasite and the proportion of infested animals for each ectoparasite species were sharply divergent in such cases as *Polygenis gwyni, Haemolaelaps glasgowi* and *Dermacentor variabilis.*

The most plausible explanation is that the cotton rats from Hillsborough County were trapped in a much drier terrain than those in the Everglades (Figure 2). Flea larvae conceivably have difficulty in developing in soil that is too moist or periodically inundated. Ticks and mites, on the other hand, probably do not find such conditions as adverse, since they pass all but the egg stage largely on the sanctuary of their host, or, if molting on the ground, are capable of extended locomotion to favorable environments.

If the animals caught in the Everglades and Hillsborough County are compared without including ectoparasites that were absent in either one of the regions, a curious result presents itself. Cotton rats in the Everglades have more than twice as many ectoparasites as do those from Hillsborough County, although the number of infestations is the same. Rice rats in Hillsborough County have twice as many ectoparasites as do rice rats in the Everglades, and the number of infestations is half again as great in the more heavily infested group. It would appear that for some reason the cotton rat is a favored host in the Everglades, being the carrier of more than five times as many individual ectoparasites as rice rats in the same environment, despite a similar pattern of host infestations. In Hillsborough County cotton and rice rats living side by side show little difference in numbers of ectoparasites harbored, although host infestations run 50 per cent higher in rice rats.

In both regions the rice rat tends to harbor but few individuals of each species of ectoparasite infesting it, whereas the cotton rat, if parasitized at all, is likely to support a larger population of each kind of vermin. This may be due to differences in the qualities of the hosts' blood, microclimatic variations in the fur of the two animals, differences in the structure and texture of the hair and skin or alternative techniques in grooming behavior. Cotton and rice rats, maintained in identical cages in the laboratory, are noticeably different in the matter of cleanliness. Cotton rat cages become foul much more rapidly than do those of rice rats and may therefore become more favorable for the presence or breeding of ectoparasites. It is possible that this observation may extend to the nests of the wild animals.

Comparisons of maximum infestations, and of average infestations per parasitized animal in these two series, show maxima in favor of cotton rats in nine out of 10 possible instances, and higher average infestations in cotton rats in eight out of 10 instances.

SUMMARY

1. This paper presents an account of ectoparasites recovered from small mammals in Everglades National Park and in Hillsborough County, Florida.

2. Differences in ectoparasite faunae of the two regions appeared to depend on topographic and climatic factors.

3. *Liponyssus bacoti* and *Polygenis gwyni,* present in both regions, displayed an annual fluctuation in their numbers, being most populous in late winter and spring. They had wide host ranges, although they were most common on cotton rats.

4. Parasitic mites generally abounded in moister environments than *Dermacentor variabilis,* although this tick in turn appeared to require more moisture than the flea, *Polygenis gwyni.*

5. Some ectoparasites tended to occupy specific stations on their hosts.

6. Cutaneous reactions of wild rodents to ectoparasites were absent in most instances. Lice caused faint pigmentation, chiggers an oozing of serum, and larval ticks small depressed scars.

7. Degrees of parasitization of individual hosts were highly variable.

8. Associations of various species of ectoparasites on a single host showed no pattern indicating an ecologic relationship among parasite species.

9. Cotton rats were more heavily and more frequently parasitized than rice rats.

10. Rice rats, harboring fewer ectoparasites of each species, were likely to be parasitized by a larger number of species per host than were cotton rats.

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THE COMPARATIVE GROWTH CYCLE OF DIFFERENT STRAINS OF *ENDAMOEBA HISTOLYTICA* IN TRANSPARENT MEDIUM AND OF THE SAME STRAIN IN DIFFERENT MEDIA*

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INTRODUCTION

Many investigations have been undertaken in the attempt to provide a better understanding of the factors responsible for the growth of *Endamoeba histolytica in vitro*. In order to evaluate the culturability of different strains of this ameba in the same medium, Faust and his co-workers (1946) cultured stools from 18 infected persons. Although no numerical determinations were carried out, these workers reported marked differences in population growth among the different strains. Since the bacterial flora present in these amebic cultures varied considerably, it was impossible to determine whether the differences in population growth were due to intrinsic properties of the amebae or to their accompanying bacterial flora.

The cultivation of *E. histolytica* in a transparent medium without demonstrable bacterial growth has been reported by Shaffer and Frye (1948). This medium appears to be ideally suited for the study of factors influencing the growth of *E. histolytica,* since very little, if any, multiplication of bacteria appears to take place in it.

The present study was carried out in an attempt to trace the growth of different strains of *E. histolytica* in this medium with an inhibited monobacterial flora and to compare it to that that in standard media with a multibacterial flora.

MATERIALS AND METHODS

Our strains 19 and 22, and NRS of *E. histolytica* were used in these experiments. Strain 19 was isolated on November 30, 1946 from a symptomatic case. Its original bacterial flora was eliminated by means of antibiotics and replaced by organism *"t"* (Faust et al, 1948). Strain 22 was isolated from a human case of amebic brain abscess on September 8, 1947 and obtained through the courtesy of Dr. J. C. Swartzwelder, Louisiana State Medical Center. To make sure that we were always employing definite genetical entities, a clone was isolated from each of the three strains by a modification of the technic described by Topacio (1933) for isolating single trypanosomes. The procedure was as follows: Strips of cellophane, $1'' \times 2''$ in size, were kept for a few minutes in 85% alcohol, rubbed between the fingers to remove the outer varnish, washed in distilled water and autoclaved. Employing sterile technic, such a strip of cellophane was transferred to a 50×75 mm. slide previously coated with a drop of serum fixative. The cellophane was made perfectly smooth by rolling a glass rod over it, and dried with a sterile gauze. With a bacteriological loop several drops from a culture of the amebae to be isolated were taken at different

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levels and deposited upon the surface of the cellophane. The slide was then placed on the mechanical stage of a microscope and observed with a 16 mm. lens. As soon as a drop containing a single ameba was found, a small square was made with a sharp knife around the drop and the cellophane paper containing the drop was removed and immersed in a Wassermann tube containing preconditioned medium warmed at 37° C. The operation was repeated several times until a large number of tubes were thus inoculated. These tubes were observed after 48-hour incubation at 37° C. and transfers were made from those in which amebae were growing. After three passages in Wassermann tubes the amebae were transferred to regular 16 × 150 mm. test tubes and routinely maintained by transfers every 48 hours.

Growth of the three clones was studied in the following media: (1) Strains 22, 19 and NRS in transparent monophasic (Shaffer and Frye, 1948); (2) Strain 22 in buffered egg-yolk infusion (Balamuth, 1946); (3) Strain 22 in inspissated whole egg slant with buffered saline overlay, and (4) Strain 22 in inspissated whole egg slant with saline and 0.5% liver extract (Lilly) overlay. The last two media were employed with and without the addition of specially milled powdered rice generously furnished by Dr. C. W. Rees, Laboratory of Tropical Diseases, National Institutes of Health. For brevity, the above media will be designated respectively as follows: S-F; B1; SES; LES. The simplified procedure was used in the preparation of the Shaffer-Frye medium (1949). Each tube of substrate contained 1.25 ml. of normal saline, 0.25 of merthiolate-preserved horse serum, 500 U. penicillin in 1 ml. of normal saline and 2.5 ml. of the supernate of a centrifugalized 24-hour culture of "streptobacillus" in BBL thioglycollate #136. Subsequent to inoculation with 0.5 ml. of a 72- or 96-hour amebic culture containing a known number of amebae, sterile petrolatum was carefully layered onto the surface and the cultures were then incubated at 37° C. The inoculum was prepared by first immersing the tubes in ice water for a few minutes to free the amebae from the sides of the tube. They were then centrifugalized for 5 minutes at 1800 r.p.m., the supernate was removed to another vessel and the amebae in the sediment counted. By dilution of the sediment with the supernate the desired number of amebae per ml. was obtained. A weekly examination of the "streptobacillus" by a Gram stain was done to detect possible contaminants.

For the other media, each experimental tube containing 5 ml. of medium received 0.5 ml. of inoculum from a thoroughly mixed 48- or 72-hour culture. The inoculum was obtained by pooling together and centrifugalizing 48- or 72-hour cultures that had been conditioned through several passages in the same medium. The number of amebae in the sediment was counted and by dilutions with the supernate the desired number per ml. was obtained. The bacterial flora in these cultures had been found to consist of 5 species of aerobic non-motile, gram-negative rods, provisionally allocated to the alcaligenes group, and of one species of a gram-positive micrococcus Carrera and Faust, 1949). When growth in S-F medium was studied, cycles were followed through ten 96-hour periods of incubation. When growth in the other media was studied, from 2 to 10 separate growth cycles were followed in each case for each size of inoculum used. All experiments were carried out in duplicate or triplicate, and at least two tubes were counted at each time interval. The amebae were enumerated on Spencer bright-line hemocytometers. After thorough shaking to insure a uniform distribution, four counts, each covering nine counting squares,

were made from each tube and the mean number per ml. was calculated. In order to avoid changes in population growth resulting from shaking of the media, the cultures were discarded after counts were made and tubes which had been left undisturbed were used for each of the following counts.

RESULTS

1. *Growth in Shaffer-Frye medium.* The data on the population growth of amebae in this medium have been summarized in table 1. This indicates that both strains 19 and 22 followed approximately the same population growth curve. The greatest increase in population occurred during the first 48 hours from the time of inoculation. Further increase in population seemed to take place in some experiments up to 96 hours but at a much reduced rate. Strain NRS was originally provided through the courtesy of Dr. W. W. Frye who brought it to this laboratory

TABLE 1.—*The mean growth of clones of different strains of* E. histolytica *in S–F medium*

Strain	Number of amebae $\times 10^{-3}$ per ml. of medium			
	At inoculation (0 hours)	After 48 hours	After 72 hours	After 96 hours
19	0.8	8	8	9
22	0.8	9	9	9
NRS	0.8 or more	gradually decreased in number until all cultures became negative		

TABLE 2.—*The population growth of clone 22 in different media with and without the addition of starch*

Type of medium	Number of amebae $\times 10^{-3}$ per ml. of medium											
	From 48-hour cultures						From 72-hour cultures					
	At inoc. (0 hrs.)	After 18 h	After 24 h	After 36 h	After 48 h	After 72 h	At inoc. (0 hrs.)	After 18 h	After 24 h	After 36 h	After 48 h	After 72 h
SES	1	7	9	10	9	3	1	4	8	12	13	9
LES	1	5	9	13	9	3	1	4	6	12	17	16
SES + starch	1	7	13	20	15	9	1	10	13	30	21	8
LES + starch	1	7	13	15	13	8	1	5	8	21	26	13
Bl	0.8	..	6	15	26	29	..	..	..	..	..	..
Bl + starch	1	18	27	46	48	10	1	7	12	28	41	31
Bl + starch	5	..	40	74	92	50	..	..	..	..	..	..
Bl + starch	16	..	67	101	81	28	..	..	..	..	..	..

TABLE 3.—*The growth of clone 22 in S–F medium in vessels with different bottom area*

Experiment No.	No. of amebae per ml. of medium (0 hrs)	No. of amebae per ml. of medium after 72 hours	
		In tubes	In flasks
I	700	1,600	5,500
II	1,000	3,000	7,400
III	1,500	3,900	7,100
Mean	1,070	2,830	6,680

from Vanderbilt University in whole-egg slant with buffered saline overlay and in S-F medium. In the latter medium the amebae gradually decreased in number until after 5 passages the cultures became negative. Subsequently, 10 attempts were made to re-establish strain NRS in S-F medium. The results were as follows: In four attempts no amebae were seen after 72 hours, twice some were alive up to the first subculture, once some survived up to the second subculture, once some survived up

to the third subculture, once some survived up to the fourth subculture, and once some survived up to the fifth subculture. After a clone was isolated from the NRS in whole-egg slant with buffered saline overlay, several attempts were made to transfer its progeny to S-F medium. In every case the amebae decreased in population until they all died out before the third culture. In order to find out whether the inability of amebae of this strain to grow in S-F medium was due to extrinsic or inherent changes which occurred while they were routinely cultured in this laboratory, another culture of NRS was obtained from Dr. W. Balamuth, Department of Biology, Northwestern University. These amebae also failed to survive in the S-F medium. Living amebae were never observed after the second subculture.

2. *Growth of clone 22 in different media.* An examination of table 2 indicates that growth of clone 22 in each medium other than S-F, followed the typical *in vitro* bacterial pattern of growth curve. The poorest growth was obtained in the media to which no rice starch had been added. With the same initial inoculum the population growth in SES and LES was strikingly similar. The addition of powdered rice starch to these two media consistently produced greater growth. With an inoculum obtained from 48-hour cultures the maximum number of amebae was observed 36 hours after incubation. When the inoculum was obtained from 72-hour cultures the curve of growth was shifted towards the right, with a peak between 36 and 48 hours after inoculation. The best yields were obtained when Balamuth medium with rice starch was used. Likewise, with this medium there was an increase in the initial lag phase when amebae from a 72-hour culture were used as inoculum. Moreover, as had been observed in previous studies, the greater the initial inoculum up to 16,000 amebae per ml. of medium, the greater was the number of amebae at the peak of population. Also, increasing the initial inoculum caused a definite shift of the curve towards the left. When 800 amebae per ml. were used, the maximum number was found to occur between 48 and 72 hours after incubation; when 1,000 and 5,000 were used, it was between 36 and 48 hours, and when 16,000 were used, it was at 36 hours.

3. *The effect of increased surface area in S-F cultures.* Working with strain 22, one of us (Everritt, 1949) observed that the population growth in small Erlenmeyer flasks was almost ten times that in tubes which contained the same amount of medium and received the same size of inoculum. This observation suggested the possibility of investigating various physical factors which might influence the populaton growth of cultures in S-F medium. Therefore, a series of experiments was set up to determine whether the growth of *E. histolytica* in S-F medium could be enhanced by increasing the surface area, by agitation of the medium (Balamuth and Howard, 1946), or by both. In two experiments a total of twenty-four 16×150 mm. test tubes each containing 5 ml. of freshly prepared medium, received 0.5 ml. of inoculum containing equal numbers of amebae from a pool of 72-hour cultures of clone 22 in S-F medium. After introducing a small glass tube 10×30 mm. in 12 cultures, to increase the vertical surface area, all the test tubes were sealed with a layer of melted petrolatum. The cultures were then divided into four groups. Six of those with increased surface area were agitated every 24 hours, and the other six were left undisturbed as controls of the first group; six of those prepared without the enclosed tubes were agitated every 24 hours and the other six were left undisturbed as controls. Seventy-two hours after inoculation six counts were made for

every tube and the mean number of amebae for each group was calculated. The results indicated that at this time the number of amebae present in the four groups was not significantly different. It was reasoned that the lack of difference in population in the groups with increased surface area may have been due to the fact that the surface increase was vertical rather than horizontal. Therefore, three more experiments were set up to compare growth of amebae in small Erlenmeyer flasks with that in test tubes. Twenty-three ml. of freshly prepared medium were introduced into each of six 25-ml. Erlenmeyer flasks and of six 50-ml. conical centrifuge tubes. They were inoculated each with 2 ml. containing equal numbers of amebae from a pool of 72-hour cultures of clone 22 in S-F medium, sealed with sterile petrolatum and incubated at 37° C. After 72 hours six counts were made for each tube and the mean population for the two groups was compared. As shown in table 3, in every experiment the flasks contained more amebae than the tubes. The difference was found to be statistically significant. The values obtained using the *t* test are: $t_{10} = 5.11$; $p < 0.001$.

DISCUSSION

This study confirms the results of Shaffer and co-workers (1948, 1949) and provides numerical evidence that good amebic growth can be obtained in their medium. In addition, it shows that its cycle does not follow the same pattern of growth as that in other media. With amebae of both strains 19 and 22 there was a rapid growth during the first 48 hours. After that time the population remained relatively stationary up to 96 hours. Although counts were not made for longer periods, microscopic observation of the tubes suggested that the decrease in population following the maximal growth is very slow. Positive cultures have been observed up to several weeks after inoculation in this medium. In comparing the culturability of strains 19 and 22 with NRS in S-F medium, it was observed that while the former two grew at a similar rate, the amebae of the latter strain gradually decreased in number in each of many attempts until the cultures became negative. These results are even more striking, considering that it is the same NRS strain that was employed by Shaffer and Frye in their studies with this transparent medium. In view of the fact that the three strains employed were studied in a medium in which little or no bacterial multiplication appears to take place, the differences in their culturability suggests that intrinsic properties of the amebae may be the determining factor in this difference. The similarity in growth of strains 19 and 22 suggests that this may be an all or none reaction. Further experiments employing a much larger number of strains are needed to clarify this important question on the biology of this parasite.

In comparing the population growth curve of strain 22 in S-F with that in the other media, it is evident that yields obtained in this transparent medium are as high as those obtained in the diphasic media without the addition of rice powder. A 10- to 12-fold increase in population was recorded in S-F, SES and LES media. On the other hand, when rice powder was added, a much more luxuriant amebic growth was obtained in all of the media with multibacterial flora, the highest being in Balamuth's medium (up to 50-fold increase). As indicated in table 2, in every instance in which amebae from 48-hour cultures were used as a source of inoculum the population growth was more rapid than when 72-hour cultures were used. Microscopic observation of samples from 48-hour cultures, when the amebae were at the peak of their growth curve, revealed actively motile organisms. On the other

hand, observation of samples from 72-hour cultures, when the amebic population was in the phase of decline, showed sluggish and highly vacuolated organisms. Thus, is seems logical that the latter type of inoculum would produce a greater initial lag phase in the population growth cycle. Likewise, a larger number of amebae in the inocula up to 16,000 amebae per ml. produced greater yields of amebae and shifted the population peak to the left.

In studying the relationship between the surface area of culture vessels and the population growth of strain 22 in S-F medium, it was observed that, while an increase in area in a vertical direction does not significantly alter the number of amebae present after 72 hours, an increase in a horizontal direction brings about greater yields. This observation, if repeated with other strains, besides having a practical application in culturing amebae in S-F medium, provides some useful information towards an understanding of the factors operating *in vitro* for the survival and reproduction of *E. histolytica*. The observation that amebae in Balamuth's medium with a multibacterial flora grow better when the bottom area of the vessel is greater does not provide information as to whether it may be due to changes in the oxidation-reduction potential, altered growth of the bacteria and/or their by-products, or whether it prevents physical or physiological crowding of the amebae. On the basis of similar results obtained with S-F medium, where both amebae and "streptobacillus" live in an anaerobic condition and where bacterial growth is inhibited by the presence of penicillin, one may conclude that the more luxuriant growth is due, at least in part, to a better opportunity to utilize the medium and a better dispersal of the metabolites. The failure to produce better growth when the area was increased vertically may be due to the action of gravity on the products in the media or on the amebae themselves, particularly since the amebae were not observed to migrate throughout the entire length of the inner vertical column.

SUMMARY

Growth of the clones of three strains of *Endamoeba histolytica* (nos. 19, 22 and NRS) was studied in S-F medium. While amebae of the two strains (nos. 19 and 22) grew at a similar rate, those of NRS consistently failed to survive in these cultures for more than a few transfers. The population growth of strain 22 in S-F medium was compared with that of these amebae in diphasic and monophasic culture media with associated bacteria, without and with starch. It was observed that in S-F medium the growth cycle fails to follow the same pattern as that in the other media. In the former the population increased rapidly during the first 48 hours, remained somewhat stationary up to 96 hours and then decreased very slowly. In the latter, the amebic population growth curve approximated that reported for typical bacterial growth. Repeated experiments with culture tubes and flasks containing the same amount of medium and inoculated with the same size of inoculum showed that an increase in bottom surface area of the culture vessels enhances significantly the population growth of amebae in S-F medium, whereas no significant increase occurs when additional vertical surface is provided.

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EXPERIMENTAL STUDIES ON TRICHOMONIASIS: 1. THE PATHOGENICITY OF TRICHOMONAD SPECIES FOR MICE

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Experimental Studies on Trichomoniasis

The present studies on the pathogenicity of trichomonad species for mice were based on the observations of Boos (1934) who reported successful attempts to infect mice with a bacteria-free strain of *T. columbae* by the intra-abdominal, subcutaneous, and intramuscular route. The infection produced a chronic disease with characteristic lesions harboring active parasites for several weeks. Boos' experiments were confirmed by Wagner (1938) who was able to reproduce the trichomonad peritonitis in mice; multiple injections of culture were required in his experiments, and it is not explicitly stated that pure cultures were used. Only a few further investigations as to the pathogenicity of trichomonad species for laboratory hosts are recorded in the literature and most of them describe unsuccessful attempts to infect lower animals (Trussell and McNutt, 1941; Trussell, 1947). Morgan (1942, 1943) succeeded, however, in infecting mice and guinea pigs with *T. foetus* by the parenteral route, particularly if he used fresh material from pyometra. In our recent experiments characteristic infections of mice were consistently produced with pure cultures of *T. vaginalis, T. foetus,* and *T. gallinae.*

This work was undertaken with the ultimate intention of obtaining an experimental method suitable for evaluation of anti-trichomonad activity *in vivo.* It seems, however, appropriate to present now the results of our primary biological investigations because the observations in the trichomonad infection of mice might offer interesting technical possibilities for the study of this parasitic infection of tissues.

MATERIAL AND METHODS

Strains of trichomonas: One strain each of *T. vaginalis, T. foetus,* and *T. gallinae* was used. We are greatly indebted to Dr. G. Johnson of the Ortho Research Foundation, Raritan, N. J., who kindly supplied us with these strains.

Culturing of the strains: The strains were grown in bacteria-free cultures using Trussell's and Johnson's (1943) CPLM medium with addition of about 6% human serum. The tubes containing 8.5 ml. medium were inoculated with 0.1–0.3 ml. of a well-grown culture and incubated at 37° C. for 24–48 hours. At that time a heavy bacteria-free growth of parasites was generally obtained. Counts of the cultures carried out in the usual hemocytometer chamber with 1% formaldehyde saline as diluent showed that cultures of *T. vaginalis, T. foetus,* and *T. gallinae* contained 1–3 millions parasites/ml., although in cultures of *T. gallinae* occasionally heavier growth (4–5 million/ml.) was observed.

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Infection of mice

Albino mice from two colonies were used in all experiments. No difference of susceptibility of the two mouse strains was observed. Inocula of known parasite count prepared if necessary by diluting the original or centrifuged cultures were injected into the mice by the intra-abdominal, subcutaneous, and intramuscular route. The volume injected was 0.5 ml. Since the trichomonad infection of mice is a strictly localized disease which did not kill the animals (only the intra-abdominal injection of *T. foetus* was fatal, if high infective doses were given), the mice were sacrificed at regular intervals from three days to twelve weeks, the lesions examined, and presence or absence of active trichomonads determined by culture and by microscopic examination of fresh or stained smears. After it had been found that the results of culturing corresponded to the microscopic findings, culturing from the lesions was not done regularly. Absence of concomitant bacterial infections was also ascertained by the usual bacteriological methods.

EXPERIMENTAL RESULTS

Intra-abdominal infection

The susceptibility of mice to the intra-abdominal injection of trichomonad culture was tested in groups of three to twelve animals which were observed for different periods and sacrificed for macroscopic and microscopic examination. Experiments with *T. gallinae* confirmed the observations of Boos. A single intra-abdominal injection of 2,000,000 parasites produced an infection for a period of at least eight weeks after an incubation period of about two weeks. The findings in the mice at the time they were sacrificed were quite uniform. The surface of the serosa was less smooth than in normal animals on account of a light fibrinous exudate. Multiple small abscesses were found in the mesenterium, sometimes between adherent intestinal loops. These abscesses which were frequently of only pinpoint size were also present in the neighborhood of the bladder and rectum. In the omentum and in the region between liver and spleen larger abscesses were found. More severe lesions could be produced by five infections on five sucessive days with heavier suspensions of about 12–16 millions parasites/ml. These concentrated suspensions were obtained by centrifuging the cultures and diluting the heavy sediment with serum free CPLM medium to the desired strength. After this course of infections, pathological changes of the liver were observed. In earlier stages, e.g., after two weeks, a fibrinous membrane was found covering the convexity of the liver; in later stages (3–6 weeks after the infection) abscesses of the liver might be found, similar to those described by Boos, (fig. 1).

Similar experiments with *T. vaginalis* indicated that the pathogenicity of the strain of *T. vaginalis* was also quite high. Single injections of cultures containing 4,000,000 parasites/ml. led to an infection after an incubation time of eight days during which no lesions and no parasites were found; after three weeks multiple small abscesses occurred invariably in the peritoneal cavity and numerous active parasites were present. There was no essential difference in the gross appearance of chronic peritonitis as produced by *T. vaginalis* and *T. gallinae,* but liver abscesses have not been observed in infections with the former parasite so far, even after repeated infection with 7,000,000 parasites. This might be due to the fact that the observation period in these experiments was not extended over three weeks.

The infection with cultures of *T. foetus* produced more severe infections. High infective doses of 100,000 parasites or more were fatal. The animals developed a marked swelling of the abdomen and died within two to three days. After injection of 25,000–30,000 parasites per mouse, 10 out of 16 mice died on the fifth to sixth day. At autopsy the abdominal cavity of dead and surviving mice was found to be filled with a grey, viscous, turbid fluid containing numerous trichomonads and leucocytes, (figs. 2 and 3). This trichomonad ascites was also present in all animals injected with diluted culture and sacrificed after 5–12 days. The minimal dose to produce a specific nonfatal trichomonad peritonitis was 1250–2500 parasites per mouse. These animals exhibited a lesser amount of peritoneal exudate which, however, still contained numerous parasites.

In later stages of the peritonitis and in case of mice which died overnight an occasional mixed infection with intestinal bacteria was noted. No bacteria were present in the majority of animals and no mixed infection was observed in the infection with *T. gallinae* and *T. vaginalis*.

The experiments on the duration of an intra-abdominal infection with *T. foetus* are not yet finished.

Intramuscular infection

All three species of *Trichomonas* produced large abscesses if injected intramuscularly, confirming the description by Boos in his experiments with *T. columbae*. Two to three days after the inoculation into the muscles of the hind leg a small but well defined swelling could be felt in the tissues. This initial infiltration increased gradually in size until a large abscess was formed after 14–18 days, (fig. 4). These abscesses, measured in three dimensions with a caliper, had a size of 500 to 2200 $mm.^3$; they were filled with thick greenish pus containing very numerous parasites and leucocytes. Abscesses developed after infection with *T. foetus* had a tendency to perforation after 6–8 weeks which was not so consistently observed after infections with *T. vaginalis* and *T. gallinae*. The muscle abscesses after infection with these species of *Trichomonas* showed reduced size after 8–12 weeks with concentration and thickening of the exudate in which, nevertheless, motile parasites persisted. The size of the inoculum had a definite influence on the duration of the intramuscular infection. The injection of 2,500,000 *T. vaginalis* per mouse produced abscesses of at least four weeks' duration, while injections with 250,000 parasites per mouse did as a rule not last longer than two weeks. No abscesses were observed after injection of 25,000 parasites per mouse. Heavy intramuscular infections with *T. gallinae*, e.g., 2,000,000 parasites per mouse, produced abscesses of more than twelve weeks duration, which still contained great numbers of active parasites at this time. Injection of 260,000 parasites per mouse was followed consistently by abscesses after two and four weeks while 26,000 parasites per mouse led to abscess formation after four weeks with an incubation period of two weeks.

T. foetus was not titrated to the endpoint. The infective dose of 850,000 parasites per mouse produced large abscesses with numerous parasites after two weeks, lasting for at least eight weeks with frequent perforation as mentioned before.

Mixed infections with bacteria did not occur after intramuscular injection of trichomonad cultures.

Subcutaneous infection

Similar to the intramuscular route, the injection of trichomonad cultures into the ventral subcutaneous tissue of mice brought about local abscess formation with all three species of *Trichomonas*. The subcutaneous route was eventually selected for the method of evaluating anti-trichomonad activity *in vivo*. Since these experiments were not extended over a period longer than five days, the ultimate duration of the subcutaneous infection was not studied, but titrations of the required infective doses were carried out with examination of the mice between three to eight days after the infection.

The results are given in Table 1 which shows that inocula of 25,000 parasites and more were sufficient to produce trichomonad abscesses of three to eight days duration with all three species of parasites. In case of *T. vaginalis* the titration was carried out to the ineffective dose of 1000 parasites. Higher doses, up to 7000 parasites, produced late abscesses after 5–8 days, while in earlier stages no active parasites were found. Similar conditions were mentioned in case of the intra-abdominal infection.

TABLE 1.—*Subcutaneous Infection of Mice with* T. gallinae, T. foetus, *and* T. vaginalis

	Single Infective Dose.		
Trichomonad species	Number of parasites × 1000	Parasites in abscesses after	
		3–4 days	5–8 days
gallinae	4,825	3+	4+
	482.5	3+	4+
	48.25	3+	3+
	4.83	3+	2 to 3+
foetus	500	3+	3+
	370	3+	2+
	100	3+	+
	50	+ to 2+	+
	25	+ to 2+	+
vaginalis	1,000	4+	–
	500	3+	–
	50	4+	4+
	25	4+	4+
	12.5	2–3+	2–3+
	7.0	0	3+
	5.0	0	3+
	3.0	0	3+
	1.0	–	0

The subcutaneous injection of cultures was consistently followed by a topical lesion which was palpable as a well defined infiltration eighteen hours after the infection and increased gradually in size to form an abscess which was clearly visible as a protuberance over the level of the skin. The abscesses tended to perforate after 10–14 days. Figure 5 is a smear taken twenty-four hours after the infection with *T. vaginalis,* and shows the presence of characteristic lumps or clusters of parasites surrounded by leucocytes. In later stages the parasites might outnumber the white cells to a considerable degree.

The infected area appeared, in tissue sections taken after four days, as a circumscribed subcutaneous abscess, (fig. 6). There was also a marked leucocytic infiltration of the connective tissue and the muscles surrounding the abscess. In sections made on later occasions, e.g., nine days after the infection, the area of the abscess was larger, involving the cutis and the abdominal muscles. The cavity of the abscess contained great numbers of parasites and leucocytes in the marginal parts, while

the central parts were more or less necrotic. Bacterial contamination did not occur.

As mentioned before, the subcutaneous infection with trichomonads was selected for the evaluation of therapeutic effects exerted by topical application of chemotherapeutic agents. The reason for this was that easily accessible infective foci could be produced with a high degree of consistency. In a large number of experiments untreated control animals never failed to show the characteristic lesion and the corresponding presence of large numbers of parasites. With an infective dose of 500,000 parasites of *T. vaginalis* all of 252 mice were heavily infected. The same result was obtained in 198 mice infected with 1,000,000 parasites of *T. gallinae* and in 48 mice infected with 200,000 parasites of *T. foetus*. Another technically important feature of these infections was their characteristic susceptibility to chemotherapeutic agents allowing the comparison of compounds of unknown activity with the effect of suitable standards of reliable effectiveness.

It might be mentioned here that we were unable to produce trichomonad infections in rats which is in accordance with findings of Morgan.

DISCUSSION

The experiments described in the experimental part confirmed the findings of Boos according to which trichomonads of avian origin produced purulent lesions of specific character in mice. Moreover, evidence was brought forth that identical infections were obtained with one strain each of *T. vaginalis* and *T. foetus*. The latter organism appeared as a rule more virulent than *T. gallinae* which in turn impressed us as being more pathogenic for mice then *T. vaginalis*.

The chronic tissue infections caused by injection of pure cultures of trichomonads were characterized by pyogenic inflammation with leucocytic cell reaction and an abundant proliferation of parasites. The purulent nature and the comparatively long duration of the experimental disease made it comparable to the chronic infections occurring in animals and man.

No explanation can be offered why attempts of other investigators to transfer trichomonads into mice were unsuccessful. It might, however, be due to the fact that in many instances natural portals of entrance were used such as the oral or the vaginal route, while injections into the serous cavities and deeper tissues are undoubtedly more favorable. It might also be that chronic non-fatal infections were overlooked because lesions might frequently develop only after a certain incubation period as was the case in some of our experiments. Furthermore, clinical symptoms particularly after intra-abdominal infection can be absent except in the case of *T. foetus*. The third possibility that not all strains of these trichomonad species possess pathogenicity for mice cannot be discussed at the present time because only one strain of each of the three species was used so far in our experiments.

SUMMARY

Evidence is submitted that one strain of each *T. vaginalis, T. gallinae,* and *T. foetus* was pathogenic for mice and produced characteristic lesions following the injection of bacteria-free cultures by the intra-abdominal, intramuscular and subcutaneous route. The duration of the experimental disease was dependent on the infective dose and lasted after higher doses for more than 8–12 weeks. Fatal infections occurred only after intra-abdominal injection of cultures of *T. foetus*.

The subcutaneous infection of mice with trichomonads was used for the evaluation of chemotherapeutic agents; these experiments will be reported in a forthcoming publication.

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Explanation of Plate 1

Fig. 1. Four abscesses in the liver of a mouse four weeks after the first of five injections of 8,000,000 parasites (*T. gallinae*) on five successive days.

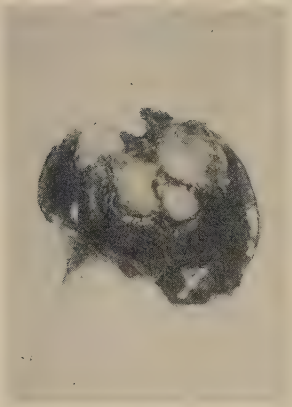
Fig. 2. Trichomonas ascites six days after intra-abdominal infection with *T. foetus* 20,000 parasites/mouse. Giemsa stain. Magnification: 170×.

Fig. 3. Higher magnification (385×) of the same slide as in Figure 2.

Fig. 4. Abscess of the leg two weeks after intramuscular infection with approximately 1,000,000 parasites (*T. vaginalis*).

Fig. 5. Smear from the subcutaneous tissue of a mouse infected with 500,000 parasites (*T. vaginalis*) twenty-four hours earlier. Giemsa stain. Magnification 385×.

Fig. 6. Section of the abdominal wall four days after subcutaneous injection of 1,000,000 *T. gallinae*. Hematoxylin—Eosin. Magnification 15×.



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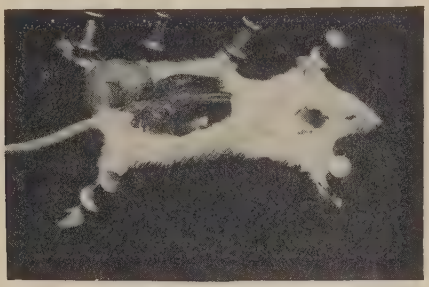

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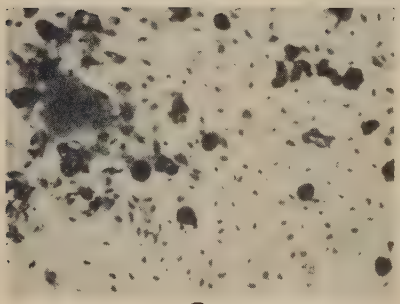

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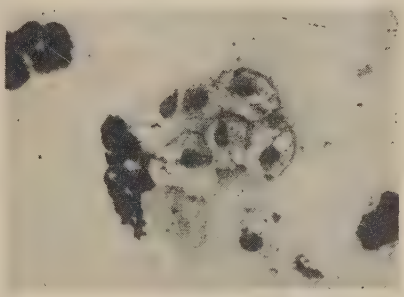

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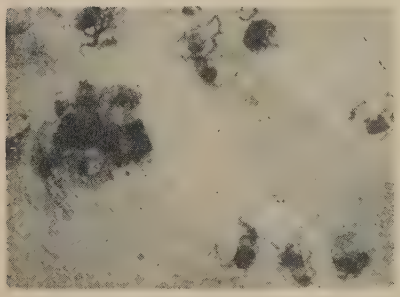

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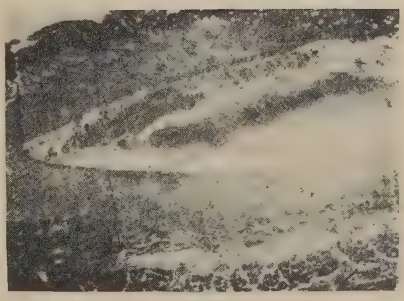

6

TWO NEW SPECIES OF *TRICHURIS* FROM NORTH AMERICA, WITH REDESCRIPTIONS OF *TRICHURIS OPACA* AND *TRICHURIS LEPORIS* (NEMATODA: APHASMIDIA)[1]

JACK D. TINER

This paper is the result of a study of a collection of whipworms loaned to the writer by Dr. Robert Rausch. The material was collected from various rodent hosts in Ohio, Wisconsin, Manitoba, and Wyoming. Some of the Wyoming collecting was done by Mr. Merle Kuns, Dept. of Biology, Purdue University.[2] Additional specimens for comparison were loaned by Dr. C. E. Mickel and Mr. Reino S. Freeman, Division of Entomology and Economic Zoology, University of Minnesota, St. Paul, Minnesota. This Minnesota material was obtained through the cooperation of Dr. F. B. Adamstone, Head, Department of Zoology, University of Illinois. Dr. Harold Manter, University of Nebraska, Lincoln, loaned specimens of *Trichuris* from a Kansas cottontail rabbit, and Barker's types of *Trichuris opaca*. Dr. E. W. Price forwarded to the writer some specimens of *T. opaca* from Maryland muskrats, and also a vial of whipworms collected from a cottontail rabbit at Bowie, Maryland, by Mr. Allen McIntosh. Thanks are expressed to Dr. Asa C. Chandler for many suggestions and a rather extensive correspondence relating to *Trichuris leporis*.

The collection studied included the specimens reported from *Tamias striatus* by Rausch and Tiner (1948) as "*Trichuris* sp." Inasmuch as no species description which fits these specimens has been found, they are regarded by the writer as new, and the name *Trichuris madisonensis* is proposed for them. The specimens reported from *Citellus richardsoni* by Rausch and Tiner (1948) as "*Trichuris* sp." have been identified by the writer as *Trichuris leporis*. Hall (1916) commented that the variation reported for the length of the spicule of *T. leporis* suggested the possibility of a "confusion of species." The writer is convinced that at least two different species have been included under this name. The male of one of the included species has a relatively short spicule (about 1.6–3.2 mm) and the writer restricts *T. leporis* to this form, which in North America occurs in snowshoe hares. The male of the second species which has been included with *T. leporis* has a relatively long spicule (6–8 mm); it has been well described by Chandler (1930). Apparently it is host specific for cotton tail rabbits. The name *Trichuris sylvilagi* is here proposed for it.

Trichuris madisonensis n. sp.

Male: Length: 21.5 mm; esophageal region 14.3 mm; thick portion of body 6.9–7.2 mm. Width of head about 0.012 mm; of mid-esophageal region 0.073 mm; at junction of esophagus and intestine 0.203 mm; rear portion of body 0.286–0.305 mm. Spicule 0.83–0.95 mm long; 0.026–0.029 mm wide; 0.100 mm from end; 0.034 mm wide in middle of shaft; and 0.033–0.036 mm wide at base. Spicule sheath, covered with spines, extends 0.052 mm beyond rear of body. Spicular diverticulum leaves cloaca 0.52–0.62 mm anterior to rear of body, at, or a little behind middle of

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[1] A contribution from the Department of Zoology, University of Illinois, Champaign, Illinois.

[2] All specimens from Wyoming were obtained while the collectors were financed by grants in aid from the New York Zoological Society's 1948 Research Program at Jackson Hole Wildlife Park.

cloaca. Cloaca 1.14–1.22 mm long. Ejaculatory duct 1.8–1.9 mm, and vas deferens 3.8–4.0 mm long. Testis with about 20–21 complete lobulations, with origin near proximal end of cloaca, about 1.22–1.44 mm from posterior end.

Female: Length 25.7–28.5 mm; esophageal region 17.2–18.5 mm; thick portion of body 8.5–10.0 mm. Vulva posterior to junction of esophagus with intestine; transverse, and without markedly prominent edges. Ovejector about 1.45 mm long. Eggs 0.072–0.075 mm × 0.033 mm. Rectum about 0.196 mm long. Anus about 0.039–0.059 mm from posterior end.

Type host: Tamias striatus.

Location: Caecum.

Locality: South edge of Lake Mendota, Madison, Wisconsin.

Type specimens: U. S. National Museum Helminthological Collection No. 37211 (holotype male and allotype female); No. 37213 (paratype male).

Remarks: The specimens from *Tamias* were first thought to be identifiable as *Trichuris muris,* but failure of Rausch (unpublished data) and of Schiller and Morgan (1949) to find this species in rats or mice at Madison made such an identification unlikely. Furthermore, it differs from *T. muris* as described by Cerecero (1943) in having larger eggs and a spicular diverticulum nearer to the middle of the cloaca.

The writer has been informed (personal correspondence, Dr. E. W. Price, 1948) that there are no specimens identified as *T. muris* from North America in the U. S. National Museum Helminthological Collection. Various persons who have examined large numbers of rats for helminth parasites (e.g. Dr. L. J. Thomas, Dr. G. F. Otto) have informed the writer in conversation that they have not found this species north of Mexico. Until specimens become available, reports of the occurrence of *T. muris* in the United States cannot be verified.

Trichuris opaca Barker and Noyes, 1915

Specimens collected from the vole *Microtus p. pennsylvanicus* and the muskrat *Ondatra z. zibethica* in Wisconsin were compared with Barker's type material, and corrections needed to make the original description apply accurately to the types were given by Rausch and Tiner (1949). A redescription based on the ample series of specimens collected by Rausch now follows. Measurements refer to specimens from the muskrat at Madison, Wisconsin, except that a note in parentheses is used when *Microtus* material from the identical locality exceeded specimens from the type host species.

Male: Length 21.8–24.9 mm; esophageal region 13.1–14.5 mm; thick portion of body 8.7–10.4 mm. Width of head about 0.010 mm; of mid-esophageal region 0.098–0.102 mm; at junction of esophagus and intestine 0.160–0.170 mm; of rear portion of body 0.286–0.305 mm. Spicule 1.20–1.36 mm long; 0.020 mm wide 0.050 mm from tip; 0.020–0.026 mm wide in middle of shaft; and 0.036–0.046 mm wide at base. Spicular sheath usually extends about 0.100–0.150 mm beyond rear end of body, where it ends in center of ellipsoidal expansion about 0.160–0.180 mm long (Fig. 4). Total length of spicule sheath about 0.180–0.250 mm. Sheath and proximal half of expansion densely covered with small spines; these become shorter and more sparse before being replaced beyond equatorial region of expansion by longitudinal ridges about 0.013 mm long. Spicular diverticulum leaves cloaca 0.230–0.360 mm from rear of body. Cloaca 1.7–2.1 mm long (Fig. 3). Ejaculatory duct 2.1–2.6 mm, and vas deferens 4.0–5.5 mm long. Testis with about 24–28 complete lobulations, originates near proximal end of cloaca, about 1.70–2.10 mm from posterior end (Fig. 3).

Female: Length 21.9–23.7 mm (gravid specimens from voles 29.2–40.9 mm); esophageal region 14.3–15.1 mm (specimens from voles 17.1–23.6 mm); thick portion of body 7.6–8.6 (otherwise incomplete, gravid specimens from muskrats measure 12.6 and14.3 mm, and from voles 17.3 mm). Ratio of thick to esophageal portions of body about 1 : 1.7 to 1 : 1.9 (specimens from voles 1 : 1.3 to 1.7). Width of head about 0.011 mm; of mid-esophageal region 0.095 mm; at junction of esophagus with intestine, 0.150–0.232 mm; and of posterior portion of body 0.436–0.500 mm. Vulva about 0.050–0.200 mm posterior to junction of esophagus with intestine, roughly circular in outline and without marked prominence. Ovejector about 0.5 mm long (0.70–0.95 mm in specimens from voles). Eggs 0.059–0.062 × 0.029 mm (0.061–0.065 × 0.029–0.033 mm in specimens from voles). Rectum 0.255–0.430 mm long. Anus usually subterminal, occasionally terminal.

Hosts: Ondatra zibethica cinnamominus (type), *Ondatra zibethica zibethica,* and *Microtus pennsylvanicus pennsylvanicus.*

Location: Caecum.

Type locality: Not specified, but possibly vicinity of Lincoln, Nebraska, 1915.

Other localities: Wisconsin, Ohio, Wyoming, and Maryland.

Type specimens: Lectoholotype male and lectoallotype female, U. S. National Museum Helminthological Collection Nos. 37204 and 37205 respectively; syntypes 37206.

Remarks: Inasmuch as Henry (1931) recorded a spicule length of 2.1 mm for the *"T. opaca"* which he reported from *Myopotamus coypus,* his identification is not consistent with the type specimens, and his species should be designated *Trichuris* sp. until it is restudied.

Trichuris leporis (Froelich, 1789)

As previously stated, the writer regards the available specimens from *Citellus* as *T. leporis.* They have been found to be indistinguishable from some of the specimens so identified by Dr. A. B. Erickson. The host of Erickson's specimens was a snowshoe hare taken at "Breinson, Minnesota." In the males the spicule ranges from 1.6 to 2.2 mm in length. The spicule sheath is provided with a terminal swelling. Following is a description of the specimens from *Citellus.*

Male: Length about 19–21 mm; esophageal region 12–12.3 mm; thick portion of body 7.4–8.7 mm. Width of head about 0.016 mm; of mid-esophageal region 0.131–0.145 mm; at junction of esophagus and intestine 0.203–0.215 mm; of rear portion of body 0.340–0.400 mm. Spicule 2.05–2.33 mm long; 0.005–0.007 mm wide throughout most of length, and 0.016–0.023 mm wide at base. Spicular sheath extends 0.130–0.420 mm beyond rear end of body and is usually swollen to a maximum width of 0.055–0.070 mm near end. Terminal portion of sheath pear-shaped. Very small spines less than 1.0 μ long cover sheath except near its distal end (Fig. 2). Spicular diverticulum begins near middle of cloaca, 0.510–0.800 mm anterior to posterior end of body. Diverticulum 0.9–1.3 mm long. Cloaca 1.7–2.1 mm long (Fig. 1). Ejaculatory duct 4.3–5.72 mm; vas deferens 1.47–2.1 mm long. Testis with about 27–33 complete lobulations, originates 0.600–1.25 mm from the posterior end, and runs forward free of lobulations for 1.0–1.65 mm.

Female: Length 17.4–20.9 mm; esophageal region 13–15.4 mm; thick portion of body 4.2–5.5 mm. Width of head 0.016–0.019 mm; of mid-esophageal region 0.140–0.145 mm; at junction of esophagus and intestine 0.174–0.196 mm; and maximum width of posterior portion of body 0.540–0.581 mm. Vulva at junction of esophagus and intestine, circular in outline, and without marked prominences. Ovejector much convoluted, 1.09–1.20 mm long. Eggs measure 0.060–0.065 × 0.029 mm. Ovary 0.326 mm from rear end, attached posteriorly by a ligament. Rectum about 0.294 mm long, anus subterminal.

Host: Citellus richardsoni.

Location: Caecum.

Locality: Garland, Manitoba.

Specimens: U. S. National Museum Helminthological Collection No. 37207.

Boughton (1932) described specimens of *Trichuris leporis* from the snowshoe hare in Manitoba which had a spicule length of 2.88 mm. Specimens from snowshoe hares at the following localities in Minnesota had spicule lengths of: Pine County, 3.2 mm; St. Louis Co., 2.65 mm; Lake Alexander, 2.50 mm. Further study will be needed to determine whether the spicule lengths of male *Trichuris* fall into groups which vary significantly between geographic localities.

Trichuris sylvilagi n. sp.

Synonyms: *Trichuris leporis* Hall 1916 (in part). *Trichuris leporis* of Chandler 1930. *Trichuris leporis* of Erickson 1947.

Male: With the morphology described for *"Trichuris leporis"* by Chandler (1930).[3]

[3] Dr. Chandler recently made a detailed study of a male collected from *Sylvilagus* in Pennsylvania and has sent to me (1949 personal communication) the following emendations to his (1930) description: ". . . the pear-shaped enlargement at the end of the spicule sheath, though apparently having a smooth contour, when examined with high power shows traces of very minute plaques representing rudimentary spines. . . . in this specimen the spicule lies in the spicule sheath where it belongs, but the cloacal tube above the junction with the spicular tube is S-shaped as . . . described . . . . In the 14th line of . . . p. 204 'spicular sheath present' should read 'spicular tube present.'"

Type locality: Beltsville, Maryland.

Type host: Sylvilagus floridanus mallurus.

Type specimen: U. S. National Museum Helminthological Collection No. 41213.

Remarks: The reports of *"Trichuris leporis"* in cottontail rabbits by the various authors cited in Erickson's (1947) paper probably refer to *T. sylvilagi.* The specimens which Erickson recorded from "southern Minnesota" cottontails apparently are the *T. sylvilagi* which the writer found in the University of Minnesota collection. These consisted of several vials from *Sylvilagus floridanus* (evidently *S. f. mearnsi*) at Preston, Minnesota.

Chandler's (1930) description of the male of "*T. leporis*," here adopted for *Trichuris sylvilagi* n. sp., is based on specimens he borrowed from the U. S. National Museum Helminthological Collection; he accepted the identification which had been made by a worker in the Bureau of Animal Industry. The writer has been informed that although these specimens cannot be located with certainty, since the Catalogue No. assigned to them has not been recorded, in all probability they were collected from cottontail rabbits at Bowie, Maryland, which is not far from the locality of the specimen which has been designated as type. The type specimen fully agrees with Chandler's emended description.

To facilitate differentiation of trichurids of North American rodents and lagomorphs, the following key has been prepared:

Key to species of *Trichuris* occurring in
North American Rodents and Lagomorphs.

1. Spicule long and slender, usually 0.010 mm or less wide throughout most of length 2
   Spicule less than 2.1 mm long, and over 0.015 mm wide 3
2. Spicule 1.6–3.2 mm long ..... *T. leporis* (Froelich 1789).
   Spicule 6–8 mm long ..... *T. sylvilagi* (this paper).
3. Eggs longer than 0.085 mm 4
   Eggs 0.075 mm or less 5
4. Vas deferens of male twice as long as ejaculatory duct ..... *T. peromysci* Chandler, 1946.
   Vas deferens of male much less than twice as long as ejaculatory duct
   T. neotomae Chandler, 1945.
5. Length of esophageal and thick portions of body of male approximately equal 6
   Esophageal portion of body of male twice as long as thick portion 7
6. Spicule 0.9–1.15 mm long ..... *T. perognathi* Chandler, 1945.
   Spicule more than 1.5–1.7 mm long ..... *T. fossor* Hall, 1916.
7. Spicule less than 1 mm long 8
   Spicule more than 1.1 mm long 9
8. Eggs 0.072–0.075 mm long ..... *T. madisonensis* (this paper).
   Eggs 0.059–0.061 mm long ..... *T. muris* (Schrank, 1788).
9. Eggs 0.059–0.065 mm long, spicular diverticulum posterior to middle of cloaca
   T. opaca Barker and Noyes, 1915.
   Eggs 0.074–0.075 mm long, spicular diverticulum anterior to middle of cloaca
   T. citelli Chandler, 1945.

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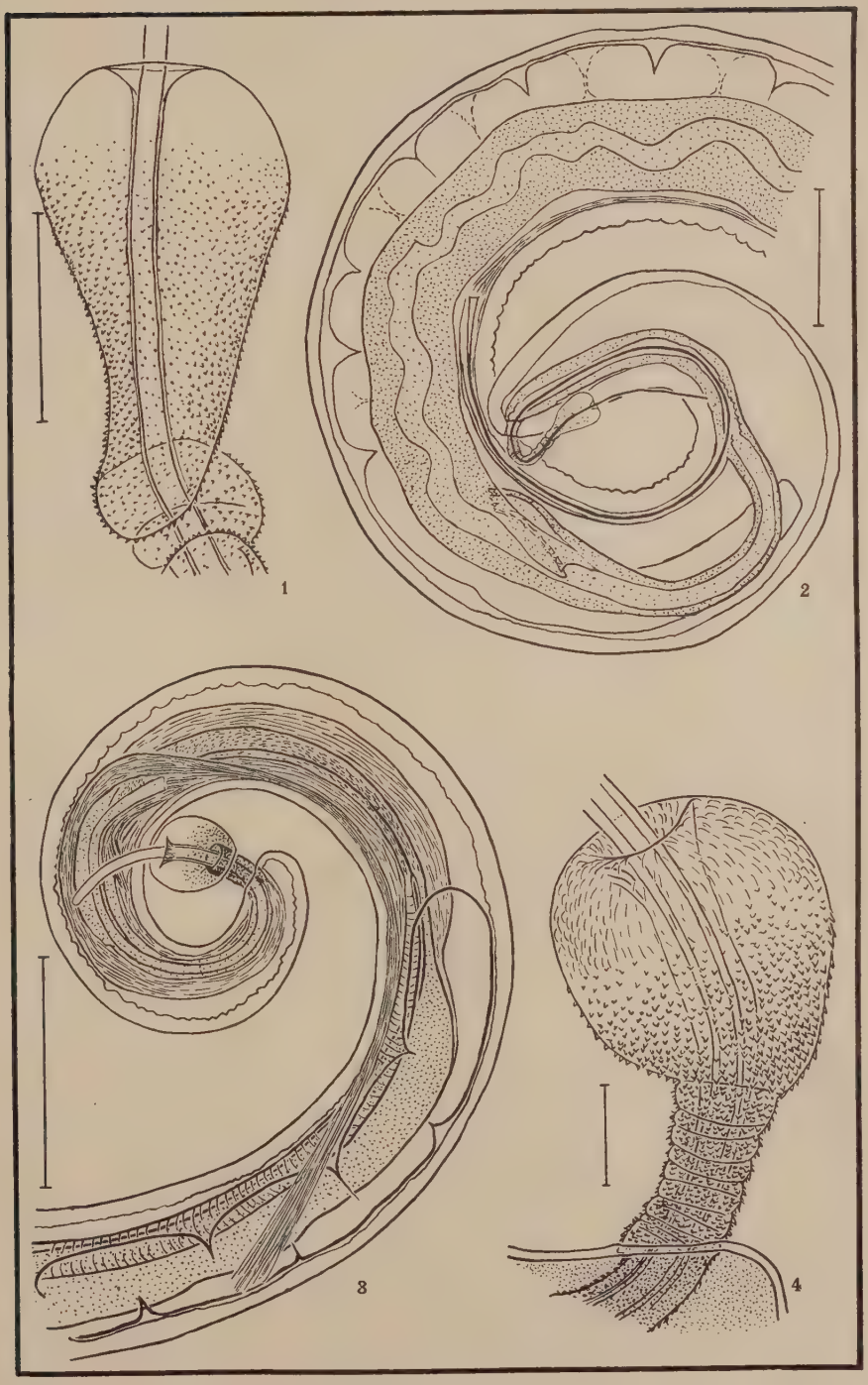
EXPLANATION OF PLATE

Scale for Fig. 1 represents 0.050 mm; Fig. 2, 0.300 mm; Fig. 3, 0.500 mm; and Fig. 4, 0.050 mm.

FIG. 1. Pear-shaped swelling at end of spicule sheath, *T. leporis* from *Citellus richardsoni.*
FIG. 2. Posterior end of male, *T. leporis.*
FIG. 3. Posterior end of male, *T. opaca.*
FIG. 4. Spicule sheath of *T. opaca.*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A NEW GENUS AND SPECIES OF TROMBICULID MITE FROM BURMA (ACARINA)*

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Among the genera of the subfamily TROMBICULINAE Ewing 1929 in which the sensillae are not markedly expanded distally, the monotypic genus *Tecomatlana* Hoffman, 1947* is unique in that there are only three scutal setae. The United States of America Typhus Commission collected in Burma a chigger which also has only three scutal setae but which is nevertheless abundantly distinctive. It is here described as a new genus.

Trisetica gen. nov.

As in *Tecomatlana* Hoffman, the posterior scutal setae are not on the plate. Separated from *Tecomatlana* in that the palpal claw is trifurcate, not with four prongs, and in that the chelicerae lack minute dorso-apical teeth. The prongs of the palpal claw are remarkably long in the new genus, being more than one-fourth again as long as that of *Trombicula deliensis* Walch (22 microns versus 15 microns). Sensillae subflagellate, but definitely more slender at proximal fourth than distally; suggesting a stage intermediate between the filamentous and spindle types. Chelicerae blade-like. Palpal claw with middle prong dorsal and longest. Eyes well-developed, with anterior eye larger than posterior. Dorsal plate broader than long; with posterior margin arcuate. Empodium claw-like.

Genotype: Trisetica melvini sp. nov.; no other species known.

Trisetica melvini sp. nov.

Larval Description (Figs. 1–4): *Body:* Outline subovate; dorsum and venter with fine parallel striations; length 0.54 mm.; width 0.38 mm. *Dorsal plate:* Anterior margin slightly sinuate, concave at the insertion of the anterolateral bristles, forming shoulders; lateral margins with a shallow median sinus; caudal margin very slightly concave, corners strongly rounded; punctate over most of its surface. Sensillae with short barbs on proximal portion, but apical two-thirds with longer cilia. Bases of sensillae inserted slightly posterior to midline of plate. Anterolateral setae short and plumose. Anteromedian bristle extending beyond apex of plate, plumose. Posterolateral setae as far off plate as bases of sensillae are from margin; near eyes; similar in shape and size to anteromedian bristle. *Eyes:* Well-developed; anterior eye with diameter of 17 microns; that of posterior eye 10 microns. *Chelicerae:* Acuminate, about four times as long as broad near base; with a single subapical notch. *Palpal claw:* With lateral prongs almost as long as middle prong. *Palpal tarsus:* Longer than broad at base; with four ventral bristles, three of which are sparsely plumed and the fourth shorter and virtually unbarbed; with a proximal ventral short spur-like process; with a dorsal long bristle which is apically plumed. *Palpus:* Trochanter and femur each with a dorsal sparsely plumed bristle; tibia with a dorsal naked bristle and with a ventral short branched seta. *Galea:* With a pair of plumed proximal ventral setae. *Dorsal setae:* Similar in appearance to anterolaterals; about 52 in number; the rows irregular, but approximate arrangement of the anterior rows is: 2 : 12 : 12 : 10. . . . *Coxae:* Punctate; unisetose, the setae plumose. *Sternal setae:* A pair of plumose setae between bases of coxae I and II; three such pair between coxae III, the middle one of each group of three more posterior than the others. *Ventral setae:* Approximately 36 in number; short and irregularly arranged. *Legs:* I—0.27 mm.; II—0.23 mm.; III—0.25 mm. Sensory setae as follows: I—two genualae, one microgenuala, two tibialae, one microtibiala, one spur, one microspur laterad to spur, one pretarsala. II—one genuala, two tibialae, one spur, one microspur

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* Hoffman, A. 1947. "Un nuevo género de trombidido Mexicano." Anales Escuela Nacional de Ciencias Biologicas **4**: 451–457.

slightly proximad to spur, one pretarsala. III—one genuala, one tibiala. *Tarsal claws:* Elongate, acuminate, curved, resembling the blade of a scythe; middle claw slightly thinner than others.

STANDARD MEASUREMENTS (in microns)

Slide No.		AW	PW	SB	ASB	PSB	A-P	AM	AL	PL	Sens.	DS
1032–1	Holotype	45	64	20	23	17	33	40	33	43	56	35
1032–2	Paratype	46	66	20	26	17	33		31	46	56	33
973–1	Paratype	42	81	17	23	17	40	36	26	43	50	33
973–2	Paratype	42	56	17	23	17	26	34	26	43	53	35

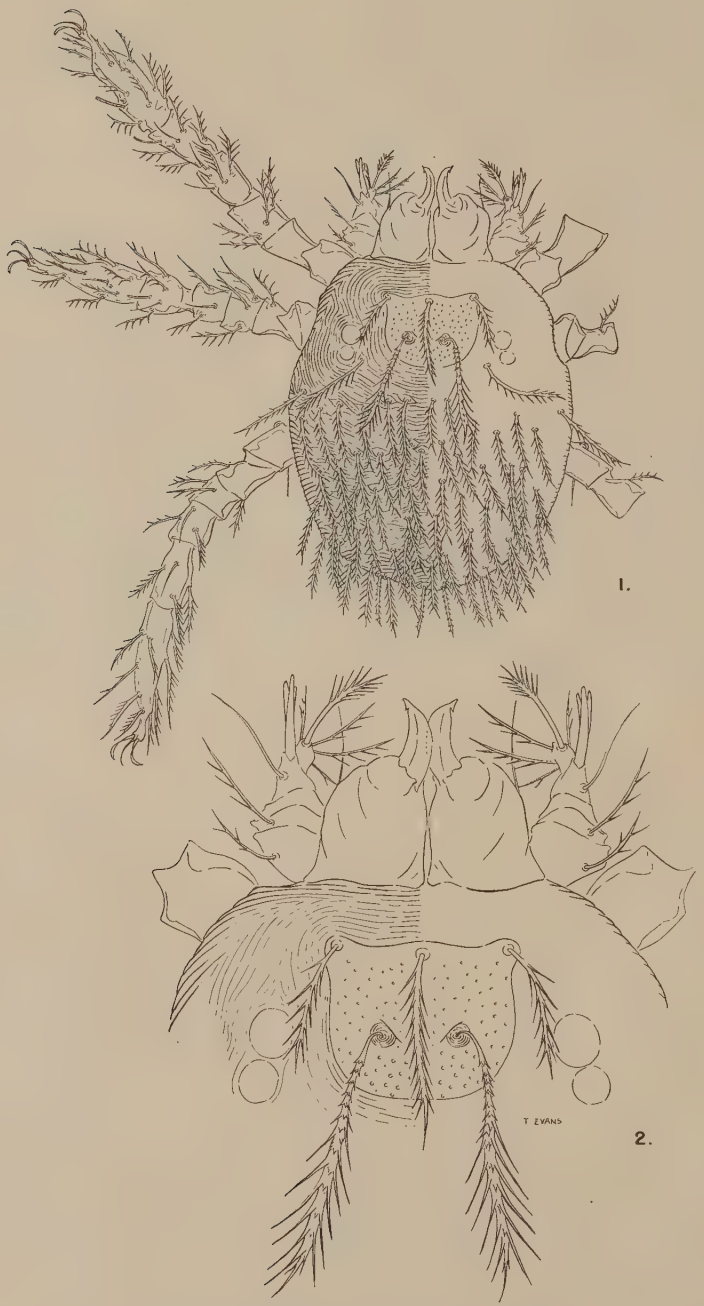
Inasmuch as the posterolateral setae are not on the scutum in this genus, a variation in PW, in ratio to degree of engorgement, is to be expected. The specimen No. 973–1 is more engorged than the others.

Type Material: Holotype (United States of America Typhus Commission No. 1032-1) deposited in the United States National Museum. Paratypes distributed as follows: The South Australian Museum, Adelaide; the Rocky Mountain Laboratory of the U. S. Public Health Service; and the Chicago Natural History Museum.

Records: Holotype and one paratype collected in petri dishes bearing paper treated with dimethyl-phthalate and exposed in a cave which had served as a charcoal-kiln, six miles north of Myitkyina, Burma, 20 May 1945. Two paratypes *ibid.*, but 1 May 1945.

Remarks: The species is named for Ray Melvin, who, while Captain in the Sanitary Corps with the United States of America Typhus Commission in North Burma, was in charge of the entomology laboratory during the very difficult early days of operation.

The cave in which these mites were collected was frequented by bats. The only trombiculid mites observed on these bats were a different species. It is of interest to note that *Tecomatlana* Hoffman, the only mite agreeing with *Triesetica* gen. nov. regarding the position of the posterolateral bristles, is a parasite of Mexican bats.



TRISETICA MELVINI GEN. & SP. NOV.
DORSAL ASPECT

FIG. 1. *Trisetica melvini* gen. and sp. nov., dorsal aspect of larva.
FIG. 2. *Ibid.*, dorsal aspect of gnathosoma and scutum.

FIG. 3. *Ibid.*, ventral aspect of larva.
FIG. 4. *Ibid.*, ventral aspect of gnathosoma and coxae I.

*PARASITICTODORA HANCOCKI** N. GEN., N. SP. (TREMATODA: HETEROPHYIDAE), WITH OBSERVATIONS ON ITS LIFE CYCLE

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INTRODUCTION

Experimental studies have demonstrated the life cycles of two pleurolophocercous cercariae which develop in the marine snail, *Cerithidea californica* Haldeman, collected from Playa del Rey, Venice, and Upper Newport Bay, California. Each has proved to be the larva of a new genus and species belonging to the family HETEROPHYIDAE. One of these, for which the name *Euhaplorchis californiensis* has been proposed, is the subject of a separate paper (Martin, 1950) while the present report is concerned with the other for which the name *Parastictodora hancocki* n. gen., n. sp. is proposed.

In a study of cercariae from the same snail collected in Upper Newport Bay, Maxon and Pequegnat (1949) found two pleurolophocercous species, one of which designated as "Pleurolophocercous I" may be the cercaria of *P. hancocki* although the description of their species is not in complete agreement with the writer's observations. However, they assigned no specific name to the larva and no taxonomic difficulties are involved, even though the two should prove to be the same species.

When exposed to cercariae, it was found that the "mud sucker," *Gillichthys mirabilis* Cooper, and the Southern California killifish, *Fundulus parvipinnis parvipinnis* (Girard), became infected with metacercariae. When infected fishes were fed to newly hatched chicks, the metacercariae excysted and developed to adults in four to six days. This species cannot be assigned to any existing genus. It therefore is necessary to erect a new one for which the name *Parastictodora* is proposed because the new genus appears to be most closely related to *Stictodora* Looss, 1899.

The writer is indebted to Dr. Carl Hubbs of the Scripps Institution of Oceanography for the identification of the fishes used in this study.

OBSERVATIONS

Parastictodora n. gen.

Diagnosis: Small lanceolate to pyriform heterophyid trematodes. Cuticula covered with small scale-like spines which decrease in size on the posterior half of the body. Anterior half of body with numerous unicellular tubular glands which open independently on the surface. Oral sucker small, prepharynx and pharynx well-developed, esophagus two to five times the length of the prepharynx, bifurcation of the intestine near the middle of body, intestinal ceca slender extending to near the posterior end of body. Genital pore median or slightly to the right of the mid-ventral line immediately posterior to the bifurcation of the intestine. A thin-walled ventrogenital sac encloses an aspinose, pyriform gonotyl provided with an irregular U-shaped, sclerotized structure and a fairly well-developed acetabulum. The metraterm and ejaculatory duct join to enter the ventrogenital sac as a hermaphroditic duct. The two, well-developed testes are very nearly side by side in the posterior third of the body; seminal vesicle bipartite, usually C-shaped, and located somewhat to the left of the median sagittal plane be-

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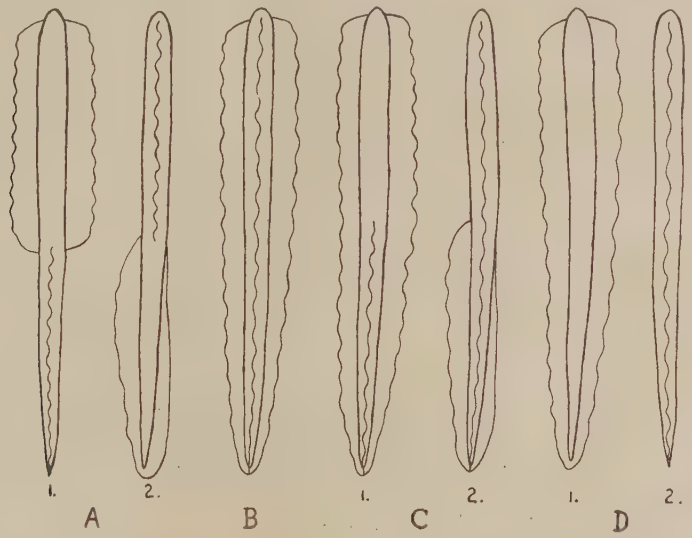
* This species is named in honor of Captain Allan Hancock, Director of the Hancock Foundation, University of Southern California.

tween the ovary and genital pore; prostate cells few in number. Ovary oval, smaller than testes, and located between the right testis and the gonotyl. Laurer's canal present; seminal receptacle medial and dorsal to the ovary, varying in size from slightly smaller to a little larger than the ovary. Convolutions of the uterus confined to the area between the genital pore and the termi-

TEXT FIGURES (diagrammatic)

Fin arrangement (1. dorsal view, 2. lateral view) on tails of A. *Euhaplorchis californiensis* and *Parastictodora hancocki* cercariae; B. *Cercaria sp.* Rothschild; C. another *Cercaria sp.* Rothschild; and D. *Haplorchis* cercariae.



nations of the ceca; eggs yellow, operculate, and without polar spines. Vitelline follicles small, diffuse, and mainly post-testicular with a slight overlapping of the testes posteriorly; main vitelline ducts converge from each side to enter a small vitelline reservoir between the testes. Excretory bladder Y-shaped, with a long stem, and extending between the testes to terminate at the level of their anterior borders. The main excretory ducts arise from the arms of the bladder, extend anteriorly to about the level of the genital pore where they divide to send secondary branches anteriorly and posteriorly; basic excretory pattern therefore of the "mesostoma" type. Larval stages develop in marine or brackish-water snails. Redia with a short gut and a birth pore; cercaria biocellate, monostomous, and pleurolophocercous. The second intermediate hosts are fishes and the natural definitive hosts probably are piscivorous birds.

Genotype: Parastictodora hancocki n. sp.

Type locality: Southern California, U. S. A.

The anatomy of the adult *Parastictodora* suggests a relationship to the genera *Stictodora* Looss, 1899, and *Galactosomum* Looss, 1899. In body shape, general arrangement of the organs and in the weak development of the oral sucker, *Parastictodora* has a greater resemblance to *Stictodora* than to *Galactosomum*. It differs from *Stictodora* in having (1) an esophagus two to five times the length of the prepharynx whereas in *Stictodora* the length of the esophagus never exceeds and usually is stated to be less than the length of the prepharynx; (2) a much better developed acetabulum which is rudimentary or possibly lacking in certain members of the genus *Stictodora;* and (3) a gonotyl devoid of spines but possessing a U-shaped, sclerotized armature whereas *Stictodora* has a spinose gonotyl. The species described by Wlassenko (1931) as *Galactosomum lacteum* may belong to the genus *Parastictodora* but its description is insufficient to decide this point.

Parastictodora hancocki nov., sp.

(Figs. 1 and 2)

Diagnosis: With the characters of the genus. Body elongate, 0.48–0.868 mm. in length by 0.149–0.298 mm. in maximum width at the gonad level. A specimen 0.48 mm. in length had

just started egg production. Remnants of eyespots may be present in the area between the levels of the oral sucker and pharynx. Oral sucker circular and subterminal, averaging 0.039 mm. in diameter. Prepharynx narrow, tubular, 0.021–0.099 mm. in length. Pharynx from cylindrical to globular in shape, 0.032–0.035 mm. in length by 0.021–0.032 mm. in width. Esophagus very long (two to five times the length of the prepharynx) measuring 0.113–0.230 mm. and tending to widen posteriorly. Acetabulum 0.031–0.033 by 0.023–0.026 mm., median to the gonotyl. Gonotyl pyriform in shape, weakly muscular, measuring 0.046–0.059 by 0.028–0.038 mm.; with a hollow, U-shaped, sclerotized structure but devoid of other armature. The ventrogenital sac receives a short genital atrium, formed by the union of the ejaculatory duct and the terminal part of the uterus, opposite the gonotyl. Right testis somewhat posterior to and consistently larger than the left, and measuring 0.053–0.124 by 0.064–0.124 mm.; left testis 0.050–0.099 by 0.056–0.113 mm. The vasa efferentia unite to form a short vas deferens which empties into a large, bipartite, thin-walled seminal vesicle which usually is bent upon itself. Terminal portion of male duct with slightly swollen pars prostatica. Ovary smooth, oval in shape, measuring 0.036–0.064 by 0.041–0.085 mm.; located about mid-way between right testis and gonotyl. Oviduct joined by Laurer's canal which opens on the dorsal surface. Seminal receptacle frequently filled with yolk granules in young worms just beginning to produce eggs; usually about the size of the ovary. Eggs numerous, at first colorless but becoming yellow with age; 20 in fixed material measuring from 0.025–0.028 mm. in length by 0.013–0.016 mm. in width with an average of 0.0267 by 0.0142 mm. (Fig. 3).

Host: (experimental) *Gallus domesticus*

Type specimen: No. 491, Parasitology Collection, Hancock Foundation.

In experimentally infected chicks, the worms were localized in the small intestine between the gizzard and the stalk of the yolk sac. They were attached by their oral suckers to the mucosa between the villi. No evidence of tissue invasion was found.

Sporocyst

The sporocyst is a simple, saccular, sausage-shaped structure measuring up to 3 mm. in length by 1 mm. in width. Its walls contain yellow to brownish pigment unevenly distributed. Within the sporocyst are hundreds of rediae of varying sizes, the older ones containing germ balls and cercariae, the youngest with germ cells only. The sporocysts, rediae, and cercariae develop in the digestive gland of the snail.

Rediae

(Figs. 4 and 5)

The mature redia is simple and has a short gut and a birth pore near the anterior end. Rediae are extremely variable in size but the dimensions of the pharynx seem to be almost constant, measurements of fifteen ranging from 0.018–0.024 mm. long (av. 0.021) and 0.021–0.025 mm. wide (av. 0.024). The redia shown in Fig. 4 measures 1.04 mm. in length by 0.13 mm. in width.

Young rediae (Fig. 5) measure 0.064–0.106 mm. in length by 0.035–0.05 mm. in width. Each possesses a well-developed gut with a narrow esophagus leading to the pharynx. Along the anterior portion of the gut and the posterior portion of the esophagus are ten gland cells with large nuclei and coarsely granular cytoplasm. The ducts from these glands extend anteriorly along the esophagus and empty into the posterior end of the pharyngeal cavity. The glands resemble the penetration glands of the cercaria. They disappear as the redia matures.

Cercaria

(Fig. 6)

The cercaria is biocellate and pleurolophocercous. The body cuticle is covered with minute spines and bears a few slender elongate projections. Body measurements of fifteen specimens fixed in Bouin's solution without pressure are: length, 0.071–0.099 mm. (av. 0.082); maximum width, 0.056–0.071 mm. (av. 0.066). The conspicuous, black eyespots are located approximately one-third the length of the body from the anterior end. Clusters of heavily staining nuclei surround the eyespots but in living material this area appears quite clear while the posterior one-half of the body contains granular gland cells. The mouth is partially surrounded by a ring which is incomplete anteriorly (Fig. 7) and in this opening there is a row of five spines whose points are directed toward the mouth opening. Anterior to these are two additional rows of 5–6 and 6–8 spines respectively which are used as scraping devices in penetrating the next host. These rows of spines are on the ventral side of a protrusible cone near the tip of which are located the exits of the penetration gland ducts. These ducts separate into four bundles of 3, 4, 4, 3 ducts

respectively as they pass anteriad dorsal to the oral sucker (Fig. 8). Unless viewed carefully under oil-immersion, the fourth duct in each of the two median bundles will not be detected. These ducts lead back on each side of the digestive tract to the cell bodies of the glands forming a cluster in the central region of the body. The penetration gland bodies partially surround a distinct oval-shaped mass of tissue which is interpreted to be the genital primordium. The oral sucker is followed by a slender prepharynx 0.005–0.013 mm. in length. The pharynx varies from spherical to oval and measures 0.005–0.008 mm. in diameter. A slender esophagus is present but the remainder of the digestive tract is not developed. The shape of the excretory bladder varies from a shallow V to oval. Its wall is composed of large cells containing refractile granules. The main collecting ducts pass forward from the antero-lateral borders of the excretory bladder to about the mid-body region where they divide into anterior and posterior branches. Each of these branches drains two sets of three flame cells each (Fig. 9) so that the excretory pattern can be expressed by the formula $2[(3+3)+(3+3)]=24$. The excretory exit tube runs down into the tail a short distance where it divides into right and left branches which open at the sides of the tail. The tail varies in length from 0.283–0.319 mm., averaging 0.30 mm. It is set in a fairly deep pocket and has a fin arrangement similar to that in the cercaria of *Euhaplorchis californiensis* (Figs. 10–12), having both lateral and dorso-ventral finfolds. The lateral fins extend posteriorly an average of 0.15 mm. and are wider than those of *Euhaplorchis californiensis*. Plicae in the lateral and dorso-ventral fins give the false appearance of supporting structures. The dorso-ventral fin begins dorsally a short distance anterior to the posterior terminations of the lateral fins and continues around the tip of the tail to terminate on the ventral side at about the level of its dorsal beginning. The maximum height of the fin is greater dorsally than it is ventrally. The cercaria swims intermittently in a zig-zag path with figure-eight movements of the tail and then sinks slowly in the water with the body downward and the tail bent at a right angle to it.

The cercaria designated as "Pleurolophocercous I" by Maxon and Pequegnat (1949) is similar to the larva of *Parastictodora hancocki* in the general shape of the body and tail and in the arrangement of body organs. However, there seem to be certain significant differences one of which is in the number of penetration gland ducts. They reported a 3-3-3-3 grouping of these ducts with a total of twelve, whereas in the cercaria of *P. hancocki* the duct arrangement is 3-4-4-3, with a total of fourteen. This might be explained by the difficulty of seeing the fourth duct in the two median bundles. Another difference, which is much more difficult to explain and which may indicate that we are dealing with distinct species, concerns the number of flame cells. Although in both instances the flame cells occur in groups of threes, Maxon and Pequegnat reported finding seventeen flame cells in one-half of the body whereas only twelve were found in *Parastictodora hancocki*.

Metacercaria

(Figs. 13 and 14)

The cercariae penetrate and encyst in tissues of the lower jaw, around the eyes, in the muscle and connective tissue near the bases of the fins, and in the brain case and coelom of *Fundulus parvipinnis parvipinnis* (Girard) and *Gillichthys mirabilis* Cooper. This cercaria has much less tissue specificity than that of *Euhaplorchis californiensis* which encysts only upon the surface of the brain of *F. parvipinnis parvipinnis*. The jack smelt, *Atherinopsis californiensis* Girard, living in the vicinity of infected snails, did not serve as a second intermediate host of *P. hancocki*. Heavy infections, particularly in *Gillichthys*, can be established experimentally. Seventy-five metacercariae were removed from one *Gillichthys* after twenty-four hours exposure to cercariae. A young metacercaria removed from the coelom of *Gillichthys* forty-eight hours after exposure, is shown in Fig. 13 enclosed in a thin, single-walled cyst. The body of this metacercaria measures 0.124 mm. in length by 0.071 mm. in width and is not flexed while in older metacercariae the body increases in size and is folded upon itself (Fig. 14). In the latter, there can be detected a number of adult structures including the ventrogenital sac, gonotyl, testes, ovary, uterine loops, and the complete digestive tract. The penetration glands disintegrate but their ducts still can be seen in the region of the oral sucker. These older metacercariae are enclosed in double-walled cysts which in the one shown in Fig. 14 measures 0.163 by 0.117 mm. In experimental infections of *Gillichthys mirabilis*, it was found that the metacercariae must age for at least two weeks to become infective for the definitive host. Also, those of minimum age to be infective mature more slowly than do those three to four weeks of age at the time of feeding. Older metacercariae become egg-producing adults within four days after feeding and have the uteri well filled with eggs by six days whereas several more days are required for metacercariae two weeks of age to attain this degree of maturity when fed to chicks.

Although *Gillichthys mirabilis* can be heavily parasitized with these metacercariae, death of the fish as a result has not been observed. If this did occur the parasite would have some economic importance since this fish is extensively used for bait by fishermen.

DISCUSSION

The adult *Parastictodora hancocki* is similar in a number of respects to the genera *Stictodora* Looss, 1899, and *Galactosomum* Looss, 1899, but its cercaria is similar to that of *Euhaplorchis californiensis* Martin, 1950. The adult of *E. californiensis* is similar to the adults of the genus *Haplorchis* Looss, 1899. Looss (1899) placed the genera *Haplorchis* and *Galactosomum* in the subfamily HAPLORCHINAE and in the light of the knowledge gained from the above life cycles it seems advisable to return to this arrangement and to suppress the subfamily GALACTOSOMINAE Ciurea, 1933. Stunkard (1946) has pointed out that knowledge of life cycles of digenetic trematodes has rendered obsolete certain of the higher taxonomic units above the family group and it is very likely that the same may be true of some of the lesser taxonomic categories. The evidence thus far indicates that the cercariae of members of the HAPLORCHINAE Looss, 1899, are oculate and possess tails with lateral fins with or without dorso-ventral fins. The lateral fins may run part of the length of the tail as in the cercariae of *Euhaplorchis californiensis* and *Parastictodora hancocki* (A) or the full length of the tail as in the cercariae of *Haplorchis* (D) and the two cercariae (B) and (C) described by Rothschild (1938) the adults of which are unknown but which are believed by the author to belong to the subfamily HAPLORCHINAE. It would be interesting to see how the adults of these two cercariae are related to the present genera and species of this subfamily. According to Price, (1940) the subfamily GALACTOSOMINAE includes the genera *Galactosomum* Looss, 1899 (synonyms: *Microlistrum* Braun, 1901; *Cercarioides* Witenberg, 1929; *Tubanguia* Srivastava, 1935), *Stictodora* Looss, 1899 (synonyms: *Cornatrium* Onji and Nishio, 1924), and *Acanthotrema* Travassos, 1928, and the subfamily HAPLORCHINAE the single genus *Haplorchis* Looss, 1899 (synonyms: *Monorchotrema* Nishigori, 1924, *Kasr* Khalil, 1932). To this list should be added the genera *Euhaplorchis* Martin, 1950, and *Parastictodora*. In the writer's opinion all of these genera should be placed in the subfamily HAPLORCHINAE Looss, 1899, and the subfamily GALACTOSOMINAE thereby be suppressed.

The distinction between certain of these genera is vague. However, *Haplorchis* and *Euhaplorchis* can be separated from the others by the presence of a single testis and can be distinguished from one another by the short, pouch-like intestine and spinose gonotyl in *Euhaplorchis* and the long ceca and absence of spine-bearing rods on the gonotyl in *Haplorchis*. Chen (1949) erected the genus *Haplorchoides* for six species formerly included in the genus *Haplorchis*. He stated that *Haplorchoides* has a longer prepharynx, shorter esophagus, and a more anterior location of the testis and vitellaria than *Haplorchis*. In the author's opinion, these characters are of questionable significance for generic distinction. It is more difficult to separate the other genera from one another. The chief distinguishing characteristics of the genus *Galactosomum* appear to be: a well-developed oral sucker, a very short esophagus never exceeding one-half the length of the prepharynx, and a solidly muscular gonotyl or gonotyls provided with spines. The significant taxonomic structures in the genus *Stictodora* are: a weakly developed oral sucker, an esophagus about equal in length to the prepharynx, and a gonotyl that is not solidly muscular. In the original de-

scription of the genus *Stictodora,* Looss (1899) stated that the pharynx was about in the middle of the esophagus. His use of the term esophagus included what we now designate as prepharynx as well as the esophagus. Yamaguti (1939) emended the genus *Stictodora,* stating that the prepharynx is long and the esophagus relatively short. However, his figures of *S. japonica* Yamaguti, 1939, *S. mergi* Yamaguti, 1939, and *S. lari* Yamaguti, 1939, show the prepharynx and esophagus about equal in length. Also Yamaguti's use of the term acetabulum for the gonotyl, and genital pad for what is very likely the acetabulum, is confusing. In fact there has been considerable variation in the terminology employed by various authors for this genital complex. The work of Cable and Hunninen (1942) may be of considerable value in bringing order to this chaos. Travassos (1928) described the genus *Acanthotrema* and placed it in the HAPLORCHIDAE although two testes are present. In most respects the description, without figures, seems to fit that of the genus *Stictodora.* He stated that the prepharynx is extremely long and the esophagus very short but his measurements of these two regions of the digestive tract of *A. acanthotrema* show that the maximum length of the esophagus is equal to the average length of the prepharynx. Most of the species of the above mentioned genera probably possess acetabula generally in a rudimentary condition but in the genus *Parastictodora* this organ is fairly well developed (Fig. 2).

Poche (1925) erected the family STICTODORIDAE which seems unjustified because it would separate the genus *Stictodora* from genera to which it undoubtedly is closely related. He also stated that there is no acetabulum in the genus *Stictodora* but Africa and Garcia (1935) reported that *S. manilensis* has a rudimentary ventral sucker. Also, Garcia and Refuerzo (1936) suggested that an acetabulum may be present in *S. guerreroi.*

It is very likely that most of the species of the above mentioned genera normally are parasitic in the intestines of fish-eating birds but like other HETEROPHYIDAE they show a conspicuous lack of host specificity. Balozet and Callot (1939) reported that the duck could act as host to *Stictodora sawakinensis.* Nazmi (1930) obtained *Galactosomum* (*Cercarioides*) *baylisi* from the domestic goose. Martin (1950) established infections of *Euhaplorchis californiensis* in chicks and Bamberger and Martin (unpublished data) in cats. Africa and Garcia (1935) and Africa, Garcia and Leon (1935) found *Stictodora manilensis* in street dogs and man in Manila. Onji and Nishio (1924) recovered *Stictodora* (*Cornatrium*) *fuscatum* from cats and *S. perpendiculum* from cats and dogs. Poirier (1886) found *Galactosomum* (*Distomum*) *erinaceum* in the porpoise. Srivastava (1935) has found *Haplorchis attenuatum, H. piscicola, H. gangeticum,* and *H. silundii* in the intestines of certain Indian freshwater fishes. Dayal (1935) found *Haplorchis* (*Monorchotrema*) *taakree* in an Indian freshwater fish. Garcia (1936) established experimental infections of *Haplorchis* (*Monorchotrema*) *taihokui* in white rats. Africa (1937, 1938 a b)., Africa and Garcia (1935), Africa, Leon and Garcia (1936 a b, 1937, 1940), and Faust and Nishigori (1925, 1926) have reported the occurrence of several species of *Haplorchis* in man, resulting not infrequently in death. Given the opportunity, it seems likely that the majority of the previously mentioned species could establish themselves in man, possibly with the serious results which have been reported by Africa (1937, 1938 a b), Africa, Leon and Garcia (1935, 1936, a b, 1937, 1940), and Africa, Garcia and Leon (1935).

SUMMARY

A new genus and species of heterophyid trematode, *Parastictodora hancocki,* are described. Adults of *P. hancocki* were obtained from chicks fed experimentally infected fishes, *Fundulus parvipinnis parvipinnis* (Girard) and *Gillichthys mirabilis* Cooper. The natural definitive hosts probably are piscivorous birds.

Sporocysts, rediae, and cercariae develop in the digestive gland of the marine snail, *Cerithidea californica* Haldeman. The cercariae are biocellate and possess tails with both lateral and dorso-ventral fins. The excretory bladder of the cercaria is sausage-shaped and the flame cell pattern is represented by the formula, $2\ [(3+3)+(3+3)] = 24$.

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Explanation of Plates and Figures

(All drawings made with the aid of a camera lucida unless otherwise stated)

Abbreviations

A acetabulum; B excretory bladder; Bp birth pore; C cyst wall; E eyespot; Eo esophagus; G pore of ventrogenital sac; Go gonotyl; Gp genital primordium; Gs ventrogenital sac; I intestine; L lateral fin; O oral sucker; Ov ovary; P pharynx; Pe penetration glands; Pr prepharynx; S seminal receptacle; Sv seminal vesicle; T testis; V vitellaria; Vf ventral fin.

Plate I

(All figures in plates concern *Parastictodora hancocki* unless otherwise stated)

Fig. 1. Ventral view of adult.
Fig. 2. Ventrogenital sac enclosing acetabulum and gonotyl with its U-shaped sclerotized armature.
Fig. 3. Egg.
Fig. 4. Older redia with developing cercariae and germ balls, showing birth pore and pharynx.
Fig. 5. Young redia.

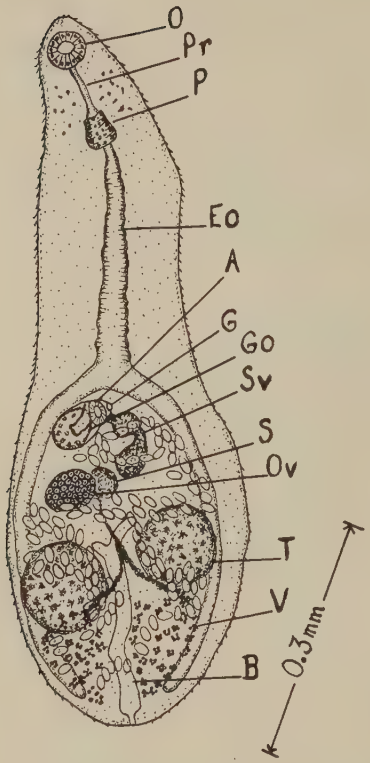
Plate II

Fig. 6. Cercaria.
Fig. 7. Diagram of incomplete ring and spines associated with cercarial mouth.
Fig. 8. Diagram of penetration gland ducts in cross section.
Fig. 9. Diagram of flame cell pattern.
Fig. 10. Cercaria of *Euhaplorchis californiensis*.

Plate III

Fig. 11. Diagram of incomplete ring and spines associated with cercarial mouth in *E. californiensis*.
Fig. 12. Diagram of flame cell pattern of *E. californiensis*.
Fig. 13. Forty-eight-hour old metacercaria.
Fig. 14. Fully developed metacercaria.

PLATE I



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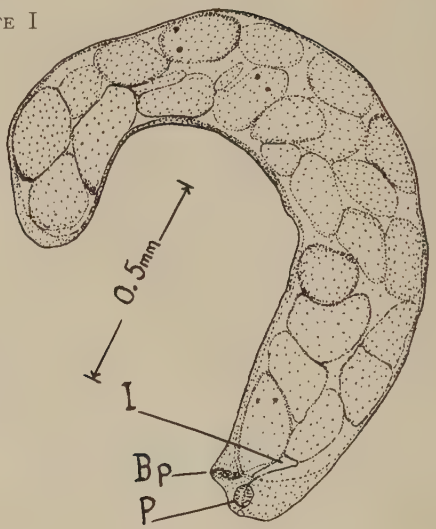

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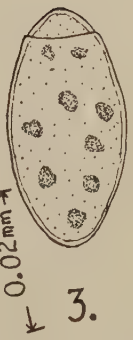

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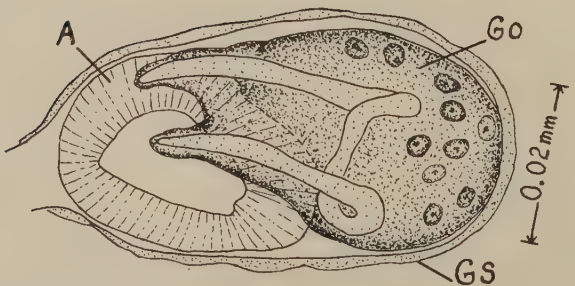

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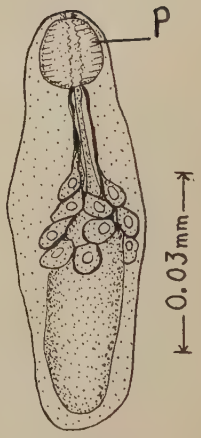

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PLATE II

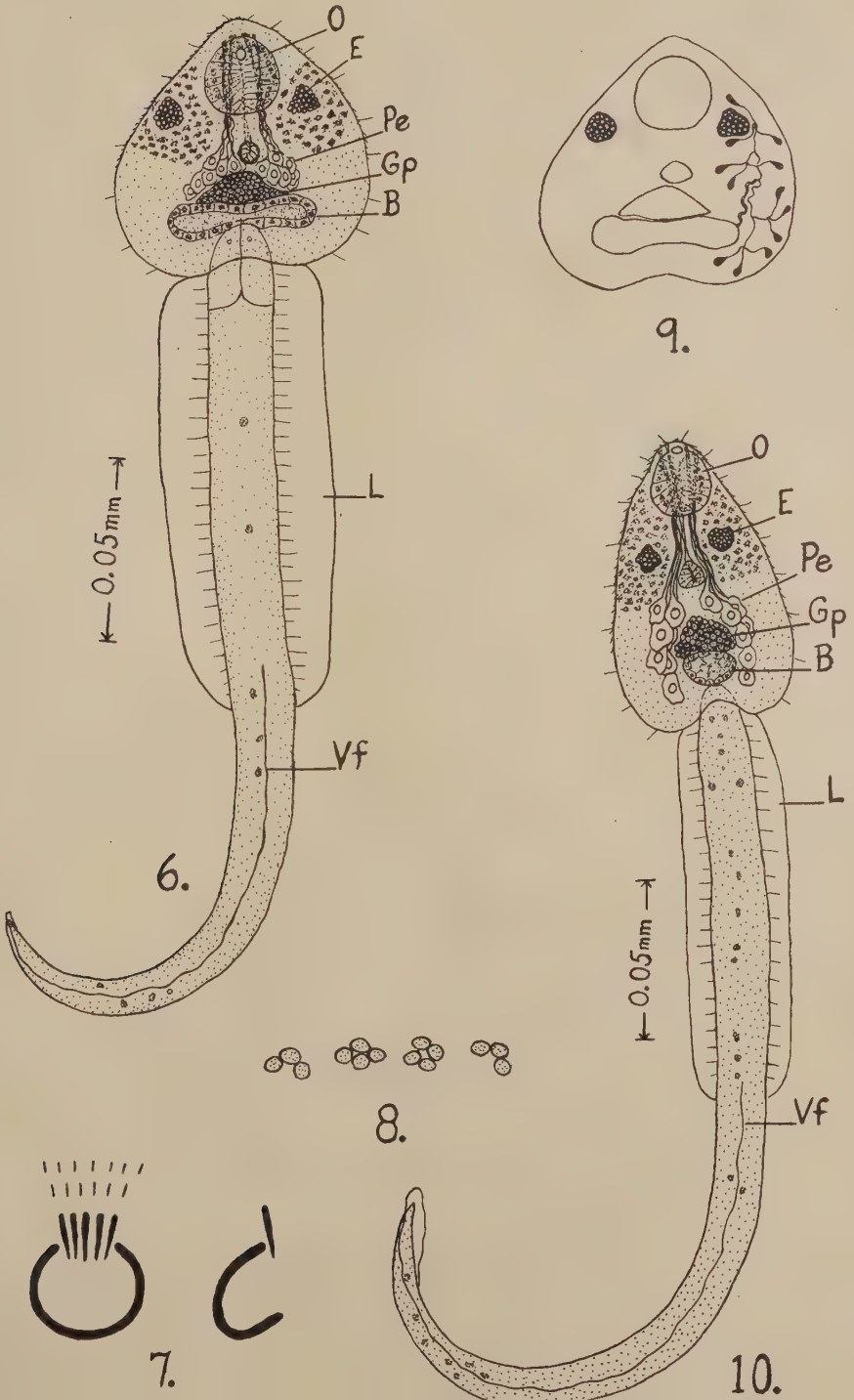
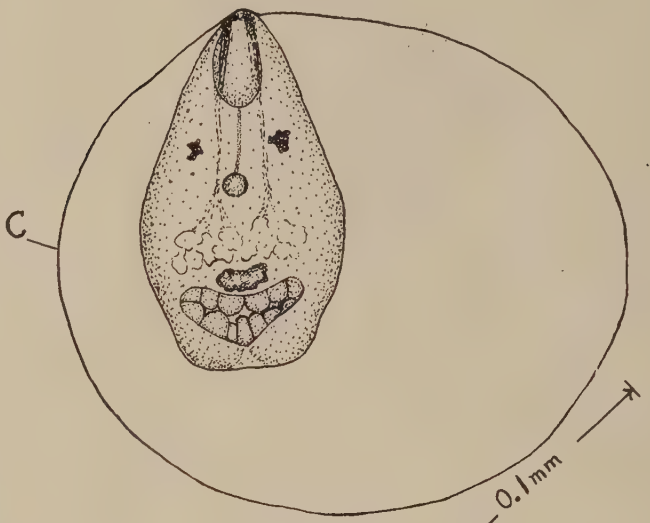


PLATE III

11.



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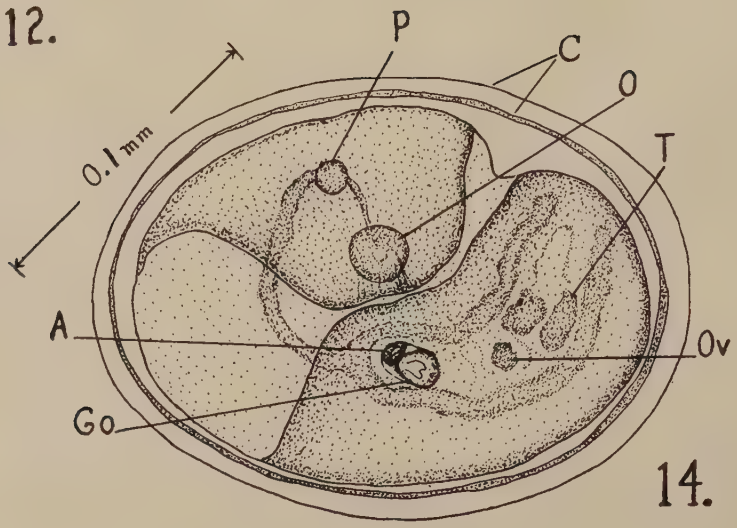

14.

STUDIES ON TAPEWORM PHYSIOLOGY. V. FURTHER OBSERVATIONS ON THE MATURATION OF *SCHISTOCEPHALUS SOLIDUS* (DIPHYLLOBOTHRIIDAE) UNDER STERILE CONDITIONS *IN VITRO*[1,2]

J. D. Smyth

INTRODUCTION

The present paper is a continuation of an investigation into the physiology of pseudophyllidean cestodes by *in vitro* methods. *Schistocephalus solidus* is a small worm whose adult stage occurs in fish-eating birds; the plerocercoid larvae occurring in the body cavity of the three-spined stickleback, *Gasterosteus aculeatus*. Previous work has shown that plerocercoid larvae may be removed aseptically from the fish host and cultured to maturity in sterile liquid media at 40° C.; 48 hours incubation at this temperature being sufficient for worms to produce eggs *in vitro* (Smyth, 1946). Eggs from these artificially matured worms, however, failed to embryonate, thus indicating that conditions of artificial culture were not sufficiently close to those existing in the bird gut (the normal adult environment) to allow normal development to proceed. Similar experiments carried out with the plerocercoids of the closely related genus *Ligula intestinalis*—also from fish—produced a similar result; unembryonated eggs being produced (Smyth, 1947a). More recent work on *Ligula* has shown that during development, acidic metabolic by-products are formed and excreted into the media resulting in considerable pH changes. When the pH was controlled by using heavily buffered media, a small percentage (6%) of embryonated eggs was obtained (Smyth, 1948, 1949).

In the light of these results with *Ligula,* a fresh approach to investigating some of the factors controlling the development of *Schistocephalus* has been made—special attention being paid to the question of egg development.

Many of the experiments described here were carried out in collaboration with C. A. Hopkins who had investigated the carbohydrate metabolism of *Schistocephalus* under different conditions of cultivation; his results will be published separately.

MATERIALS AND METHODS

Fish infected with plerocercoids of *Schistocephalus* were obtained by netting and trapping from Roundwood reservoir, near Dublin. Aseptic cultures were obtained by the dissection technique described in detail elsewhere (Smyth, 1946); large larvae, 100 mgs. or more in weight were used, as below this size the genital Anlagen are insufficiently developed. Cultivation was carried out in rimless tubes or flasks in an incubator at 40° C. ± 0.5, the average body temperature of birds (Wetmore, 1921). The following media were used; normal and ¾ strength Ringer-Locke; meat-extract-peptone-broth (Lab-lemco, 1%; NaCl, 0.5%; peptone, 1%); natural-beef-broth (juices from 2 lbs minced beef per liter of water, NaCl, 0.5%; peptone, 1%); horse serum.

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[1] From the Department of Zoology, Trinity College, Dublin, Ireland.

[2] The writer wishes to acknowledge his indebtedness to the Medical Research Council of Ireland for a generous grant-in-aid.

In order to obtain, for comparison, a number of normal worms, i.e. worms matured naturally in a bird gut, infected fish were fed to pigeons. After 48 hours, those birds whose feces were positive for eggs were killed and dissected. Some of these worms—hereafter called 'control' worms—were fixd for histological study, others were used for egg embryonation tests. 'Control' eggs were obtained from these worms, relaxed overnight in tap water, by teasing out with a fine needle. Eggs from cultured worms were easily collected as they accumulated at the bottom of the culture vessel.

This use of pigeons as experimental hosts for *Schistocephalus* was suggested by Dr. T. Kerr of Leeds University, to whom the writer makes grateful acknowledgment.

Eggs from both sources were tested for embryonation, by placing in watch glasses in about 10 cc. of water containing a trace of streptomycin (1 : 20,000) ; the presence of the latter in no way interfered with development, (Bremner, 1949), but inhibited the growth of microflora. The watch glasses were themselves contained in covered petri dishes. Testing of eggs for embryonation was carried out in an incubator at 25–26° C. Detailed development was followed by means of a high-powered water immersion lens, which enabled the progress of embryonation to be followed without removing the eggs from the watch glasses.

EXPERIMENTAL RESULTS

a. Maturation of Schistocephalus in pigeon

The time of oviposition, histology and egg embryonation of the control worms matured in the pigeon gut were studied in detail. Such observations present standards whereby the normality of the maturation *in vitro* under different conditions could then be judged. Clearly, if cultured worms required the same incubation time as control worms to reach maturity, underwent similar histological changes and finally produced eggs capable of normal embryonation, it could be concluded that development *in vitro* did not differ significantly from that *in vivo*.

Time of oviposition. Approximately 40 hours after ingestion of infected fish by the bird, eggs began to appear in the feces of the host. Eggs have, however, been found in the uterus of a worm after only 36 hours maturation. Making allowance for the fact that eggs must accumulate in sufficient quantities to be readily seen, the figure of 40 hours incubation has been taken as the approximate 'normal' incubation period required before worms become sufficiently mature to produce eggs in quantity.

Histological changes. In the plerocercoid stage, the rudiments of the testes, ovaries, yolk glands and tubular genitalia are present in all but the most anterior segments. The detailed histology has been described previously (Smyth, 1946). Maturation is extremely rapid and typical oogenesis, spermatogenesis, vitellogenesis and shell formation take place. The vagina, which in the plerocercoid is a narrow tube, grows considerably in size and develops a swollen region—the receptaculum seminis—which connects internally with the oviduct and the uterus and opens externally by a vaginal pore (Plate I, Fig. 9). This receptaculum (Plate II, Fig. 2) was always filled with spermatozoa in the numerous sections of mature worms examined. In view of the absence of spermatozoa in the receptaculum of cultured worms, this observation is highly significant. Actual fertilization of an ovum by a

spermatozoon was never observed although many hundreds of sections were examined.

Egg embryonation and hatching. Many thousands of eggs from worms matured in pigeons were tested for embryonation. Embryonation approached 100% in most cases; in a few cases a small percentage of unembryonated eggs (maximum 5%) was found. Embryonation followed the well-known pattern of the DIPHYLLOBOTHRIIDAE resulting in coracidia which almost filled the entire egg (Plate I, Fig. 1; Plate II, Fig. 3); very little yolk remained in the fully embryonated eggs. Embryonation took 7–8 days at 25–26° C.; hatching occurred in 9–10 days, releasing typical actively swimming coracidia. Development and hatching of the coracidia closely followed the descriptions given by Schauinsland (1885) and Thomas (1947).

b. Maturation of Schistocephalus in vitro

pH changes in media. The importance of the pH changes in the media has been emphasized in the case of *Ligula;* it was, therefore, the first factor considered. A large number of preliminary—and very empirical-experiments were carried out to obtain an indication of the order of the pH changes to be encountered. These experiments are too numerous to be recorded here in detail—a single experiment is quoted to indicate the extent of the pH drop. (Table I).

TABLE I.—*Comparison between the behaviour of plerocercoids of* Schistocephalus *cultured at 40° C. in vitro in saline and serum.*

Experiment No.	Medium	Culture No.	Behaviour on each day of cultivation			pH changes
			1st day	2nd day	3rd day	
5	¾-strength Ringer-Locke. 50 c.c. per tube Medium unchanged throughout	1	active	active	dead : no eggs in medium	8.0 – 4.8
		2	"	sluggish	" " " " "	8.0 – 5.4
		3	"	active	" " " " "	8.0 – 5.0
		4	"	dead : no eggs in medium	" " " " "	8.0 – 5.8
		5	"	active	" " " " "	8.0 – 5.6
		6	"	sluggish	dead : a few abnormal eggs in medium	8.0 – 5.2
27	Fresh horse serum 50 c.c. per tube. Medium unchanged throughout	1	"	active & ovipositing	sluggish	7.4 – 7.0
		2	"	" " "	active	7.4 – 6.6
		3	"	" " "	"	7.4 – 6.8
		4	"	" " "	"	7.4 – 6.5
		5	"	" " "	"	7.4 – 6.2
		6	"	" " "	"	7.4 – 6.4

Table I compares the pH changes in 6 cultures of *Schistocephalus* in 50 cc. ¾ strength Ringer-Locke (an almost unbuffered medium) with those in 50 cc. horse serum (a highly buffered medium). The maximum pH change in saline being 8.0 to 4.8 in 48 hours, the corresponding figures for serum being 7.4–6.2. In all media in which large pH drops took place, larvae either died rapidly or underwent only partial change with retarded development as indicated by histological examination. It was at once evident that large volumes of media would be required if the pH was to be maintained within a reasonable level; except in special cases, therefore, volumes of media ranging from 50–250 cc. were used in subsequent experiments. In the cases of unbuffered media i.e. saline, solid $CaCO_3$ was added—a method used for certain bacterial cultures—and which was highly successful in the present work, maintaining the pH at a high level. It was thus possible to determine whether normal development could take place in non-nutrient media, i.e. under starvation conditions. The initial technique adopted with salines was to use 200 cc. Ringer-Locke + 0.5 gr. solid $CaCO_3$. Since the $CaCO_3$ formed a heavy, white precipitate

on the bottom of the flask or tube, the eggs produced were obscured and it was found very difficult to separate them from the carbonate. The procedure finally adopted (expts. 46, 52) was to culture in saline + $CaCO_3$ for the first 30 hours and transfer to 50 cc. pure saline for the 18 hours of the 48 hours cultivation period. By this method, the eggs which began to appear at 40 hours were obtained from the bottom of the tube, quite free from carbonate.

The fall in pH during development *in vitro* was partly due to CO_2 production, but largely due to the excretion of acidic metabolic waste products into the medium by the worm. Owing to technical difficulties of extraction, these acidic products have not yet been identified, although a few milligrams of an acid crystalline substance has been obtained by continuous ether extraction of saline media used for cultivation.

General behavior in vitro. Some account of the behavior of the larvae during cultivation has already been given (Smyth, 1946). Larvae removed from the fish host showed only sluggish activity, expanding and contracting vigorously at the bottom of the culture tube. As incubation proceeded, the cirrus region of the proglottids became gradually swollen until ultimately the cirrus everted out of the cirrus pore and began its typical in-and-out movement previously described (Smyth, 1946). The cirrus is of considerable length and becomes fully extended at the zenith of the evaginating movement, waves about for a moment, ejects a cloud of spermatozoa (just visible under extremely favorable conditions of illumination—but usually very difficult to observe) and invaginates. A second or two later the process is repeated. Cirrus activity can be even more closely observed by placing an active larva—after about 40 hours cultivation—in a watch glass in warm serum and observing under low power on a warm stage. It is essential to maintain the temperature at near 40° C. or cirrus activity ceases immediately. If an active worm is further flattened between glass plates immersed in warm serum, the vigorous contraction of the seminal vesicle can also clearly be seen. If the pH is allowed to drop by using only small quantities of media, the cirrus activity either does not occur at all, or occurs spasmodically and finally ceases completely.

Development of eggs from worms matured in vitro. Once the pH was sufficiently controlled, worms *in vitro* produced eggs in all media within 40 hours, thus satisfying the first criterion for normal development as compared with the control worms.

The results of testing these eggs for embryonation are given in Table II. Three distinct types of embryos were found to occur in these eggs:—(a) 'normal'—with full-sized coracidia; (b) 'miniature'—with small coracidia; (c) 'abnormal'—with abnormal coracidia.

'Normal' coracidia never appeared in more than 5% of the embryonated eggs obtained from culture of worms in any media. These embryos (Plate I, Fig. 2) closely resembled those of control eggs (Plate I, Fig. 1) with the coracidium nearly filling the entire volume of the egg. In general, such eggs contained more residual yolk than was normally present in control eggs. These embryos moved actively within the egg and hatched out naturally without difficulty into coracidia (Plate I, Fig. 7) which did not appear to differ significantly from coracidia hatched from control eggs.

'Miniature' embryos differed from control embryos in size, amount of residual

TABLE II.—*Egg Embryonation of Schistocephalus cultured in various media under different conditions.*

Exp. No.	Volume (c.cs.)	No. of larvae	Medium	Culture vessel and conditions	Range of pH changes	Probable respiratory conditions	% of egg embryonated				Comments
							Min.	Max.	Mean = m	$\pm\sigma m$	
31	10	10	Horse serum	Tubes : Medium renewed 12-hrly.	7.6 – 7.0	semi-anaerobic	0	10	1.7	0.99	worms white
13	25	10	" "	" Medium unchanged	7.3 5.8	" "	0	15	3.9	1.66	" "
27	50	6	" "	" " "	7.4 – 6.2	" "	0	65	13.5	9.55	" "
29	50	10	" "	" " "	7.4 – 6.4	" "	0	60	14.3	5.87	" "
51	75	6	" "	" " "	7.4 – 6.8	" "	5	86	44.0	14.54	" "
8	25	8	Peptone-Broth	" " "	8.7 – 5.8	" "	0	1	0.4	0.25	" "
33	100	12	" "	Flasks : " "	8.0 – 7.1	aerobic	0	1	0.0	0.08	worms brown
35	100	12	" "	" " "	7.7 – 7.3	"	0	10	0.8	0.80	" "
36	100	9	" "	Tubes : " "	7.9 – 7.1	semi-anaerobic	0	5	0.8	0.51	worms white
37	100	10	" "	" " "	7.9 – 6.8	" "	0	0	0	0	" "
64	250	9	" "	Flasks : " "	8.0 – 7.1	" "	1	40	17.3	9.55	worms white : 3 worms per flask
63	250	9	" "	Flasks : Constant stream of air passing through medium	8.1 – 7.1	aerobic	..	8	...	....	worms in 2 sterile flasks brown : worms in infected flask white and produced eggs
62	250	9	" "	" " " " N_2 " " "	8.1 – 7.3	anaerobic	1	59	22.0	15.15	worms white
65	250	6	" " +1% glucose	Flasks : Medium unchanged	8.2 – 7.3	semi-anaerobic	5	7	6.0	0.71	" "
55	75	7	Beef-Broth + peptone	Tubes : " "	7.7 – 6.7	" "	1	56	20.2	7.28	" "
54	250	5	" " "	Flasks : " "	7.7 - 7.4	" "	4	37	20.2	5.44	" "
46	200	7	¾ Locke + $CaCO_3$	Flasks : Cultured in 25 c.c. in tubes for last 18 hrs.	8.2 – 6.6	" "	0	65	28.1	7.95	" "
26	50	4	" "	Tubes : Constant stream of air passing through medium	8.4 – 6.0	aerobic	..	..	...	....	worms brown : no eggs produced
52	200	6	N. Locke + "	Flasks : Cultured in 25 c.c. in tubes for last 18 hrs.	8.4 – 5.8	semi-anaerobic	1	51	30.2	6.86	worms white

yolk and ability to hatch. The majority of such embryos (Plate I, Fig. 3; Plate II, Fig. 4) were about half the size of normal embryos, but the size range extended from about one-third normal size up to full normal size. These miniature embryos were never observed to hatch, although they exhibited the typical undulant coracidial activity. At a stage in egg cultivation when 'normal' eggs in the same culture were hatching, 'miniature' coracidia became extremely active and by means of active ciliary movement of the embryophore rotated rapidly within the shell. The inability of such embryos to hatch was probably due to their small size preventing the embryo from exerting sufficient internal pressure to force open the operculum. It was easily possible to hatch these eggs by puncturing with a fine glass needle or pressing gently on the coverslip.

'Abnormal' embryos in most cultures made up the greatest proportion of eggs capable of development. An accurate description of these embryos is difficult as yolk was present in considerable quantity and greatly obscured the developing embryo. The most common characteristic—and the earliest to appear in development —was the possession of what appeared to be a clear vacuole (E, Plate I, fig. 4) with thick walls. This 'vacuole' proved to be the embryophore, for the cilia at a later stage, could be seen beating within it. Due to observational difficulties referred to above, the exact location of the cilia was difficult to determine. Under oil immersion, the cilia appeared to be *within* the clear cavity of the vacuole though it is more likely that they were, in fact, on its surface, and the appearance described was due to focussing phenomena. These embryophores were usually completely free from internal protoplasm and the coracidium proper—as indicated by the presence of the hooks—lay outside the embryophore area (Plate I, figs. 4 and 5). The coracidium in such eggs could only be made out by very careful focussing and in many instances the hooks were only visible through the great masses of unabsorbed yolk. Eggs containing such embryos were never observed to hatch—nor was it possible to hatch them artificially by puncturing; such a technique merely resulting in the release of a hollow, incomplete sphere of ciliated cells—the embryophore—and an amorphous mass of protoplasm containing 6 well-defined hooks. In many hundreds of egg cultures examined only one free-swimming, abnormal coracidium, which probably hatched from this type of egg, was ever observed. This consisted of a dumbbell-shaped organism (Plate I, fig. 6) with an anterior ciliated half and a posterior unciliated half, the whole enclosing a small, irregular-shaped coracidium.

As Table II indicates, the percentage of eggs undergoing embryonation varied enormously, not only in worms cultured in different media, but in worms cultured in the same media under apparently the same conditions. Thus, in the most successful experiment (No. 51) in which six larvae were cultured in six separate tubes, each with 75 cc. serum, the egg embryonation percentages were 77, 5, 20, 21, 86, 55%. Again in Exp. 29, the figures were: 17, 1, 5, 1, 20, 0, 15, 10, 60. These figures represent the general type of result obtained. In the face of such extreme variation, as indicated by the high values of $\sigma\bar{m}$, it is not possible to compare accurately the egg embryonation percentages from larvae matured in different media. It is only possible to draw the most general conclusions from such figures. On the basis of *maximum* embryonation obtained, the most suitable media would appear to be serum, $\frac{3}{4}$ Locke + $CaCO_3$, peptone-broth, normal Locke + $CaCO_3$, in that order and under the conditions of cultivation as stated; such a conclusion must be accepted only with

considerable reservation as it is based on statistically insignificant grounds and it is likely that a number of unknown factors are operating.

Effect of oxygen on maturation in vitro. In preliminary experiments, no attention was paid to the possibility that the amount of available oxygen in the media was an important controlling factor. That this, in fact, was the case became first evident on the results of experiments 35 and 36. In the former experiment, larvae were cultured in 100 cc. broth in flat-bottom flasks; this volume of media covered the bottom of the flask to a depth of about $\frac{3}{4}$"—thus exposing a very large surface to the air. Worms cultured under these conditions showed a heavy, browning effect along their edges in the region of the yolk glands. In experiment 36, the same volume of the same medium was used in tall 1" culture tubes; worms cultured under these conditions were pure white in appearance. Since worms matured in the gut of pigeons never showed any trace of this browning phenomena, it was suspected that the browning observed in worms cultured in shallow media in flasks was due to a higher O_2 tension. This conclusion was confirmed by experiments 26 and 63 in which larvae were cultured in media with sterile air (previously warmed) bubbling through; under such conditions worms became very dark in color and emitted only long strings of brown, yolky material from the uterine pores instead of eggs. In one culture (in expt. 63), through which air was passing, *white* worms and normal eggs were obtained. This latter result was puzzling, but examination of the media revealed a very heavy bacterial infection; it seems possible to explain this result on the grounds that the bacteria consumed the oxygen at a sufficiently high rate to lower the amount of oxygen available for the worm.

Worms cultured in broth under completely anaerobic conditions, i.e. in an atmosphere of nitrogen (expt. 62), did not appear to differ significantly in color from those cultured in tubes which suggests that the conditions at the bottom of the tubes was semi-anaerobic—at least to an extent that prevented browning of the yolk-glands or interference with the subsequent development.

Histology of cultured worms. In all experiments larvae were removed and stored in Bouin's fluid. When the results of testing the eggs for embryonation was known, any particular worm could then be embedded and sectioned. .The histology and cytology were studied in considerable detail in the hope that some light might be thrown on the puzzling question of egg fertility. Special attention was paid to the question of the 'giant' cells previously reported in the testes of artificially matured *Schistocephalus* and *Ligula* (Smyth, 1946, 1949). These giant cells were found to occur only rarely in larvae cultured in media in which the pH was carefully controlled. In media in which marked pH drops were found, up to five such cells were present in most testes capsules. These cells are intensely eosinophilic and there seems little doubt that they are necrotic. Their presence can, therefore, be used to indicate unsatisfactory pH control during cultivation and, in general, can be roughly correlated with very low egg embryonation figures for the production of eggs of abnormal sizes and shapes. Thus, in larvae cultured in 100 cc. broth, in tubes, these cells are of frequent occurrence and although the overall pH drop was apparently small (expts. 36, 37) their presence suggests a localised pH drop at the bottom of the tube which was not sufficiently buffered by this medium whose buffering powers are low compared with serum. Worms cultured in 50 cc., or more, of serum or in salines + $CaCO_3$ never possessed these cells in their testes.

The *receptaculum seminis* of some 50 worms was examined in detail. *In every case the cavity of the receptaculum was entirely free from spermatozoa*—nor were spermatozoa ever observed in any part of the vaginal tube, even in worms which had a percentage egg embryonation of 86% (expt. 51).

DISCUSSION

The production of acidic by-products of metabolism by *Schistocephalus* and the inhibitory nature of the pH drop thereby produced, agrees closely with the results obtained with *Ligula* (Smyth, 1949). Other workers, notably Alt & Tischer (1931) and Brand (1933) have similarly reported acid production during brief periods of cestode cultivation. It cannot be emphasised too strongly how markedly the pH drop interferes with development; other things being equal—the more perfect the buffering and mixing of the medium, the more nearly development *in vitro* approaches that *in vivo,* as judged by time of oviposition, histology of the mature worm and percentage egg embryonation. pH control must, therefore, be considered one of the most important prerequisites of successful *in vitro* cultivation of cestodes.

The evidence presented has shown that oxygen tension is likewise an important controlling factor in development *in vitro*—the browning of the worms and the absence of normally formed eggs suggesting that the yolk-glands and the shell glands are the organs most affected. The absence of browning of worms in the bottom of long tubes suggests that, although excess oxygen is definitely detrimental, normal respiratory conditions are probably only semi-anaerobic and completely anaerobic conditions are not essential. This result agrees with conditions existing in the vertebrate gut where about 5% saturation has been quoted as an average oxygen tension (Hobson, 1948).

Behavior and maximum egg embryonation of worms cultured in normal and $\frac{3}{4}$ strength Ringer-Locke did not appear to be signficantly different, which suggests that—within these limits—osmotic pressure need not necessarily be as rigorously controlled as was formerly thought necessary (Smyth, 1946). It further indicates that the food reserves of the larva are sufficient to enable it to develop under starvation conditions for at least 48 hours. Hopkins (1950) in an extensive study of the carbohydrate metabolism of *Schistocephalus in vivo* and *in vitro* has shown that although glycogen consumption is rapid, after 48 hours considerable reserves of gylcogen remain. He has further shown that larvae cultured in glucose-broth are able to utilise the glucose for their metabolism.

The question of egg fertility raises a number of puzzling problems. Consideration of these must center round the fundamental problem—how does fertilization actually take place in a worm matured naturally in a bird gut? Does the worm lie so close to the gut wall that the everting cirrus can be bent over the proglottid and can be inserted into the vaginal pore of the same or adjacent proglottids? The spatial arrangement of the vaginal and cirrus pores (Plate I, fig. 8) are such that the cirrus would have to be almost bent back on itself to make this possible. Alternatively, does the everting cirrus *in vivo* merely evert outwards directly—as it does *in vitro*—but the close apposition of the worm to the gut wall enables spermatozoa to find their way to the vaginal pores due to the building up of a high spermatozoa concentration in a confined space? Chemotaxis may here be an important factor.

Whatever the mechanism, the presence of masses of spermatozoa in the receptacula of worms matured in birds, indicates its efficiency.

Since the *receptacula seminis* of cultured worms were always devoid of spermatozoa, the conclusion must be drawn that eggs produced from such worms are unfertilized—from which it follows that any subsequent development must, therefore, be parthenogenetic. This result confirms those obtained with *Ligula* in which the receptacula of cultured worms were likewise free from spermatozoa. If this hypothesis be accepted, a number of questions immediately arise:– (a) what is the stimulus producing parthenogenetic development? (b) why do three types of embryos develop? (c) why is there such marked variation in percentage egg development in eggs from worms cultured under apparently identical conditions?

It is well known that a number of chemical substances or physical conditions can stimulate eggs to undergo parthenogenetic development and, presumably, one of these substances or conditions exist in the medium or conditions of cultivation either of the eggs or the larvae. The results of embryonation of eggs from larvae cultured in different media—though extremely variable—suggest that larvae cultured in certain media, notably serum or highly buffered saline, are potentially capable of a high degree of parthenogenetic development, but that whether they do so or not depends on the adequacy of the stimulus producing parthenogenetic development. The irregularities of the embryonation figures further suggest that whatever the stimulus may be it is probably applied at a stage where the most irregular treatment of eggs is likely to take place, i.e. during the removal of the eggs from the culture tubes and subsequent washings and transfer of eggs from watch glasses. It is possible that the sudden transfer of eggs from deep tubes of medium with a high osmotic pressure and a low oxygen tension to a shallow watchglass of water (i.e. with a low osmotic pressure and high oxygen tension) may provide the necessary stimulus. The physical process of agitation of eggs during this procedure is a further possibility.

Several alternative hypotheses could be framed. It may be that the irregularities arise during the actual maturation of worms *in vitro* and the subsequent washing processes may not play a part at all. On this view, it would be supposed that eggs produced *in vitro* may not all have the potentialities of parthenogenetic development due to genetical or cytological abnormalities induced by abnormal conditions of cultivation. It has been shown that the testes are particularly affected by pH, and yolk-glands by oxygen tension, and possibly the ova are likewise affected by these or other factors so that not all eggs are normally formed. On this view, however, it is difficult to see why a number of larvae cultured in the same media in different tubes should show marked variation in the potential egg embryonation.

Neither of these hypotheses in itself is likely to prove correct and it is possible that a number of unsuspected and unknown factors may be at work—not the least of which may be a natural variation in the larvae used in each experiment. The production of a small number of apparently more hatchable eggs *in vitro* may indicate that such eggs are, in fact, fertile but that so few sperms are used that they are not visible in histological sections. If this is the case, only the remaining two types of eggs are undergoing parthenogenetic development.

Failure of spermatozoa to reach the receptacula seminis in cultured worms is, probably, largely a physical phenomenon. Confinement in a narow tube may prove to be an essential prerequisite for successful fertilization *in vitro*. Recent experi-

ments in this direction have so far not proved successful—the main difficulty being to confine the worm in a narrow tube and, at the same time, eliminate waste acidic products at a sufficiently high rate to maintain the pH at an adequately high level.

SUMMARY

Plerocercoid larvae of the pseudophyllidean cestode *Schistocephalus* were cultured to maturity *in vitro* in liquid media at 40° C. under aseptic conditions. Histology and egg embryonation of cultured worms were compared with those of worms matured normally in birds.

As a result of acidic metabolic by-products (unidentified) excreted by developing larvae, pH of unbuffered media fell rapidly. This pH drop greatly retarded development or caused the death of the worms. Successful pH control was established by using a highly buffered medium—serum, or by the addition of solid $CaCO_3$ to unbuffered media. In such buffered media, worms oviposited within 40 hours, but percentage egg embryonation varied within the range 0–86%. Taking maximum percentage egg embryonation as criterion, serum was the most successful of the media used.

Embryonated eggs produced *in vitro* were of three types:– (a) containing 'normal' coracidia similar in size to those developed in eggs from worms matured in birds: such eggs hatched easily; (b) containing 'miniature' coracidia—about half or less the natural size: these failed to hatch naturally, but could be hatched artificially by pricking the shell; (c) containing 'abnormal' coracidia—in which the embryophore failed to develop normally and enclosed the hooked region of the embryo: this type failed to hatch naturally, nor could it be hatched artificially.

The receptacula seminis of worms matured in birds always contained large masses of spermatozoa. Spermatozoa were never found in the receptacula of cultured worms even those which produced eggs exhibiting a high percentage embryonation. It was concluded, therefore, that most eggs from cultured worms undergo parthenogenic development; eggs which produced 'normal' coracidia may have been fertilised. Evidence is presented to indicate that the oxygen tension of the medium is an important controlling factor, and that normal development requires semi-anaerobic conditions.

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EXPLANATION OF PLATE I

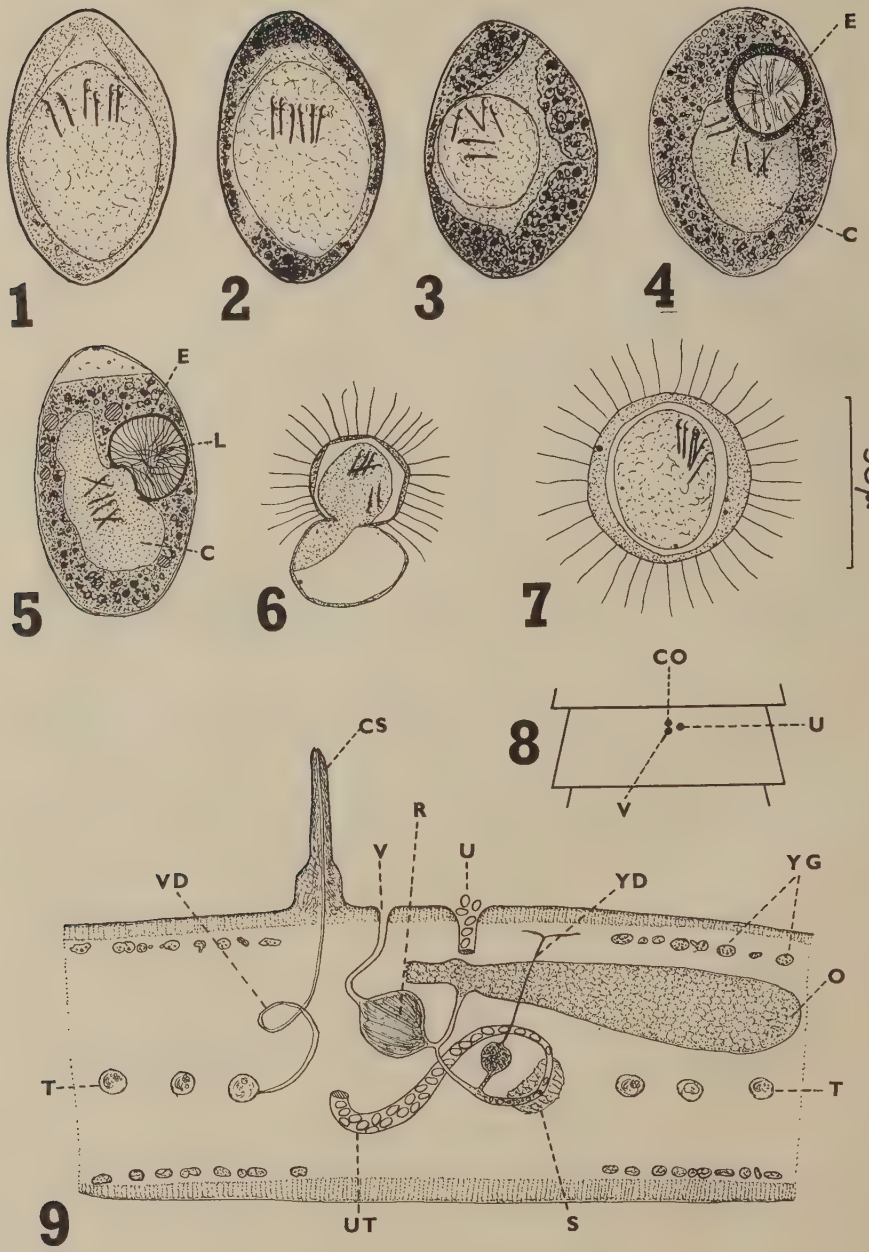
1. Embryonated egg from worm matured in bird; coracidium almost filling egg; little residual yolk.
2. Egg from worm matured *in vitro;* containing 'normal' embryo. Some yolk unconsumed, otherwise not morphologically different from 1. Probably fertilised.
3. Egg from worm matured *in vitro,* containing 'miniature' embryo: much residual yolk; development probably parthenogenetic.
4 & 5. Eggs from worm matured *in vitro,* containing 'abnormal' embryos. Embryophore almost separate from irregularly formed coracidium; probably parthenogenetic.
6. Abnormal coracidium hatched from egg from worm matured *in vitro.* This is the only specimen of this type observed.
7. Normal coracidium hatched naturally from egg with 'normal' development.
8. Proglottid of *Schistocephalus* showing arrangement of genital openings; diagrammatic.
9. Transverse section of *Schistocephalus* showing arrangement of genitalia; very diagrammatic; one ovary and part of uterus not shown.

ABBREVIATIONS

C = coracidium; CO = cirrus opening; CS = everted cirrus; E = embryophore; L = cilia of embryophore; O = ovary; R = receptaculum seminis containing spermatozoa; S = shell gland T = testis; U = uterine opening; UT = uterus; V = vaginal opening; VD = vas deferens; YD = yolk duct; YG = yolk glands.

EXPLANATION OF PLATE II

1. Section of receptaculum seminis of worm matured *in vitro* showing absence of spermatozoa
2. Section of receptaculum seminis of worm matured in bird, showing presence of masses of spermatozoa.
3. Embryonated eggs from worm matured in bird.
4. Embryonated eggs from worm matured *in vitro;* embryo in upper egg approaches normal size; that in the lower has formed a 'miniature' coracidium.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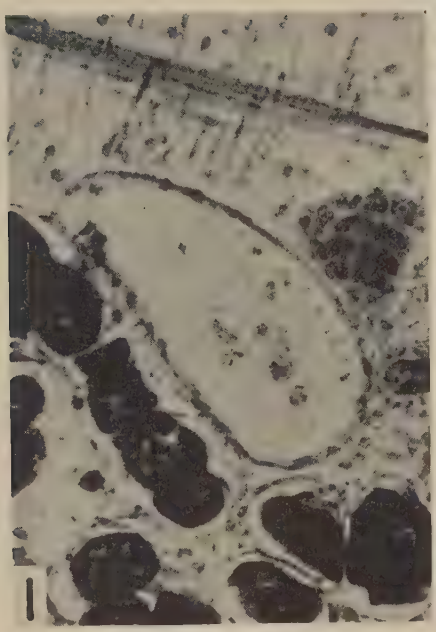
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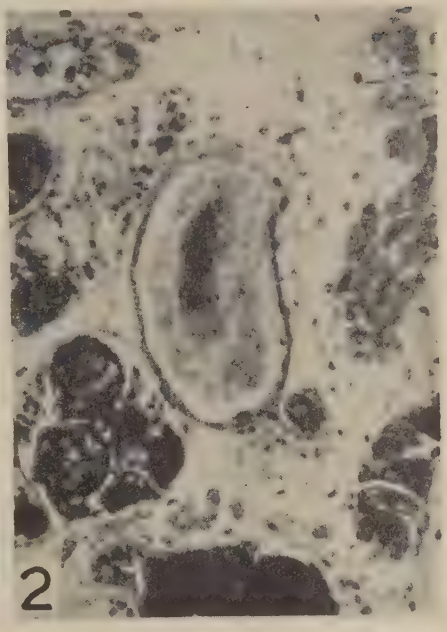
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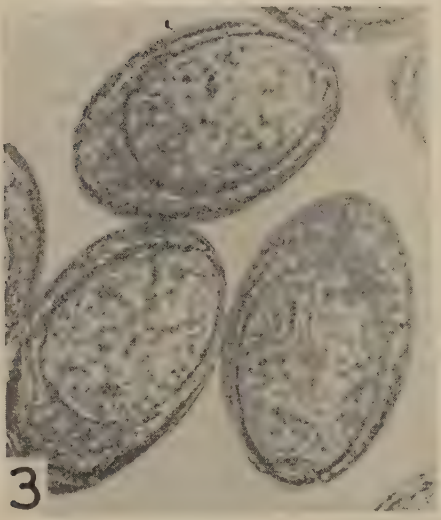
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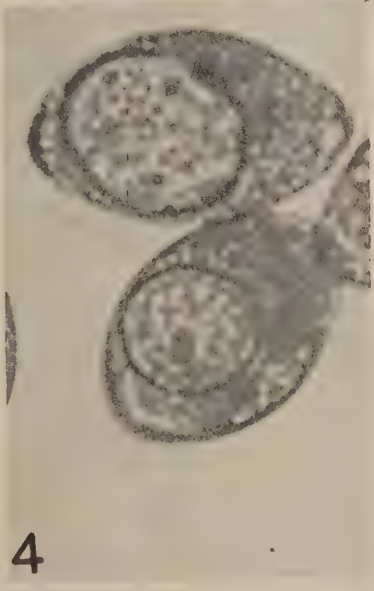
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STUDIES ON CESTODE METABOLISM. I. GLYCOGEN METABOLISM IN *SCHISTOCEPHALUS SOLIDUS IN VIVO.*

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INTRODUCTION

It has long been recognized that the elucidation of cestode metabolism, was first dependent upon the successful development of a method for culturing worms *in vitro.* The oligoseptic methods of Markov (1938) and bactericidal washings by Green and Wardle (1941) were only of a limited success. Recently both Smyth (1946), and Joyeux and Baer (1942) working independently, introduced a technique whereby plerocercoids of the *Diphyllobothriidae* could be removed and cultured under aseptic conditions.

This by itself, however, is only a technique, and the problem now remains to determine criteria by which the normality of the *in vitro* development can be measured.

It is now well established that glycogen is the main reserve food product in cestodes, and Markov (1939, 1943) used the glycogen and fat contents as criteria of starvation or nutrition. It was considered that if the rate of carbohydrate metabolism could be measured *in vivo,* during the maturation and post maturation phases, a quantitative standard would then exist, to which *in vitro* results could be compared. Divergence from this standard would indicate divergence from *in vivo,* and by definition, normal development.

Conversely if by the manipulation of the *in vitro* conditions of cutlivation, one could reproduce the *in vivo* rate, then it could be said that one had reproduced, *in vitro,* those conditions which governed cestode carbohydrate metabolism in the gut.

That the glycogen reserve is in fact a sensitive indicator of variations in metabolic conditions is well shown by the experiments of Brand (1933) and Reid (1942). The former found that in *Taenia marginata* the glycogen content was increased from 4.99% to 8.38% when the host dog was fed on a carbohydrate-rich diet. Conversely Reid found the glycogen reserves fell by over 90 per cent in *Raillietina cesticillus,* after 20 hours starvation of the host fowl.

In the present paper the initial constitution of the plerocercoid is first considered, followed by an investigation of the rate of utilization of the glycogen reserves, *in vivo,* together with notes on the distribution of the worms in the gut.

MATERIAL AND METHODS

The plerocercoids were obtained from the body cavity of *Gasterosteus aculeatus,* netted at Vartry Reservoir, Co. Wicklow.

Parasitized fish were obtained throughout the period March 1948 to October

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1949, but from November 1948 onwards the percentage infection fell to as low as one, increasing again in the spring of 1949. Similarly the plerocercoids collected during January to April were small, fresh weight rarely exceeding 120 mg., whilst in the summer and autumn of 1948 worms often exceeded 200 mg.

Fish were kept in running water tanks without feeding. Due to the possible drop in blood sugar level, and its reciprocal effect on the parasite, no fish was used after being kept for longer than nine days.

All estimations were based on fresh weight. Fresh weight was determined by removing the plerocercoids from the coelom or host gut, warming on the hands to stimulate expansion, then quickly washing in two changes of Normal Locke, drying on filter paper and weighing in stoppered receptacles.

Glycogen estimations were usually based on groups of three worms. The sample, the fresh weight of which had been determined, was hydrolzed in 2 cc. of 30% KOH, for 8 minutes, the glycogen then being precipitated with 3 cc. of 95% alcohol, heated to boiling, and allowed to stand for 15 minutes. The tubes were then centrifuged, drained and kept at 4° C. until used. This method was essentially that recommended by Good, Kramer and Somogyi (1933).

Glycogen prepared in the above way has converted into glucose by hydrolyzing for 3½ hours in N H_2SO_4. The solution was then cooled and the pH altered to between 5.5 and 6.5 by adding NaOH, using Bromo Cresol Purple as indicator. The few drops of B.C.P. did not interfere later, as the estimation was carried out at a low pH, at which after diluting the indicator was nearly colorless. The glucose solution was diluted to a convenient strength, 0.5 to 2.0 mg. %, at 20° C., and filtered. The glucose was usually measured immediately, but on occasion after storage over night in a refrigerator.

The glucose estimations were carried out by the method described by Shaffer and Somogyi (1933), using Reagent "50", with 5 grs. KI added. It was found necessary to replace the glass balls used by Shaffer to seal his reduction tubes, by rubber bungs fitted with bunsen valves. The tubes after boiling were cooled for 75 seconds in a cold water bath, and the iodine measured after 10 minutes. A burette was altered to deliver drops of 0.02 cc. at a rate of 25 cc. per 100 sec. The variation per batch of four tubes was normally less than plus or minus 0.03 cc. Figures with a greater variation than this were repeated.

Due to doubts which exist as to the correct factor, the glucose figures have not been converted into equivalent glycogen.

EXPERIMENTAL DATA

a. Chemical analysis

1. Determination of the Fresh/Dry weight ratio.

The estimations were carried out on five batches of worms. The worms whose fresh weight had been determined as described above, were killed by the addition of 5 cc. of 70% alcohol, and dried at 100° C. to a constant weight.

The mean of the five estimations, based on a total of 46 worms, of an average weight of 184 mg. was 31.78%, standard deviation 0.293.

2. Determination of Nitrogen.

Two batches of worms were used, and the estimations were carried out using the Kjeldahl apparatus in the usual way. Owing to the very high percentage of

glycogen present, the heating needed to be continued for a long time before the solution became colorless.

Percentage of Nitrogen was 1.805 and 1.840 in the two samples, the mean being 1.82%. The protein was calculated by multiplying this figure by 6.25, giving mean protein 11.4%.

3. Determination of Ash.

Two batches of seven worms each, whose average weight per worm was 127, and 145 mg. respectively, were incinerated in crucibles, to a constant weight. Their ash content was 1.75% and 1.94%, giving a mean of 1.85%.

4. Determination of Glycogen.

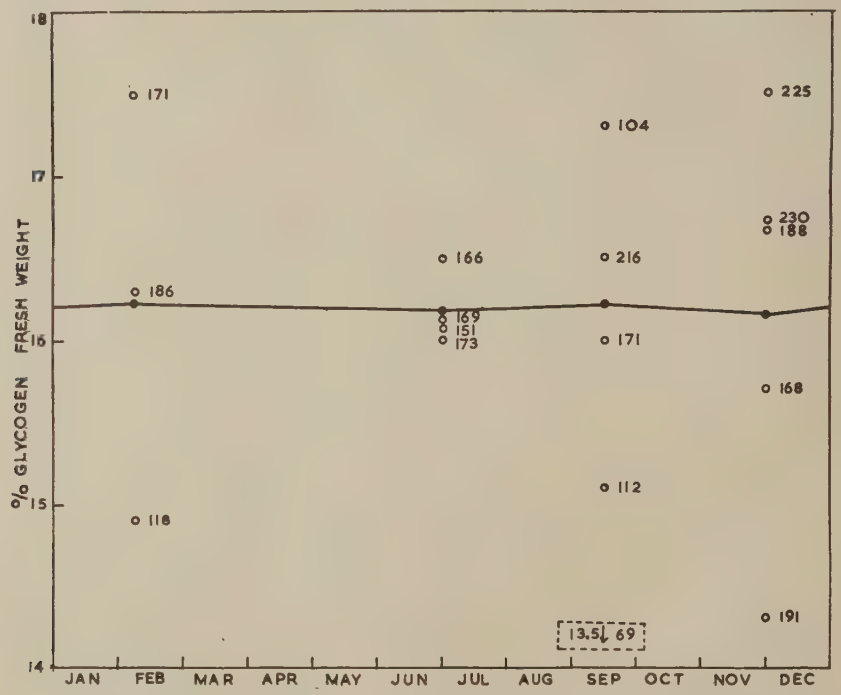

FIG. 1. Percentage glycogen content, fresh weight, of *Schistocephalus* plerocercoids during the year. Open circles—single determinations, closed circles—mean values. Numbers—mean weight in mg. of plerocercoids in sample.

In order that experimental figures could be compared throughout the year it was necessary to determine whether there was a seasonal fluctuation in the amount of glycogen stored. Figure 1 shows the result of these determinations.

In so far as conclusions may be drawn from 16 determinations the glycogen level is approximately constant. A second possible source of variation existed in the glycogen/weight ratio. It was necessary to determine whether the glycogen reserve increased in direct ratio to the increase in plerocercoid weight. From an examination of over 200 estimations and the average weights of worms used, it seemed probable that worms less than 100 mg. fresh weight had a lower percentage level of reserves than those over this weight. This is in agreement with Markov (1943),

who reported that smaller *Diphyllobothrium* plerocercoids had less glycogen. In worms exceeding 100 mg. no correlation existed. It is for this reason that the figure marked in a dotted ring on Figure 1 is not included. 100 mg. is probably a safe margin and the border line is almost certainly nearer 85–90 mg., but figures based on worms whose average weight was less than 100 mg. have not been included unless so stated.

The mean of the above sixteen estimations based on 56 worms is 16.2%, standard deviation 0.871. This figure has been taken as the initial glycogen level in all experiments.

b. Glycogen reserves during in vivo incubation.

Method.

Fish which were thought to be parasitized from external appearances, i.e. swelling of the abdomen, were pithed, spines and fins removed, and fed to pigeons. My thanks are due to Dr. T. Kerr of the University of Leeds, for suggesting the use of pigeons.) The fish were pushed into the bird's pharynx and the beak held shut until swallowed; two fish were fed per bird. With the exception of the 24 hour experiments, feces were examined for eggs before killing the birds. The eggs were plainly visible in fecal smears after 44 hours. This examination was most important to avoid needless waste of pigeons, as a large number of the worms were destroyed and no infection resulted. The feeding of *Schistocephalus,* dissected from the fish, by itself never resulted in an infection.

TABLE 1.—*Glycogen content of* Schistocephalus solidus, *after incubation in the pigeon gut.*

Hours of incubation	% Glycogen fresh weight in worms				No. of determinations	Mean % Glycogen	Standard deviation
24	11.5	12.4	11.9	11.7	4	11.9	0.335
48	10.9 9.7	8.7 11.9	10.4 11.0	10.2 12.0	8	10.6	1.032
72	11.0 9.9	9.0	10.6	9.6	5	10.0	0.711

In view of Reid's (1942) work on *Raillietina cesticillus,* in which he showed a statistically highly significant decrease in the percentage glycogen content of worms after host starvation, care was taken to see that the pigeons had food before them all the time.

After the required number of hours, the birds were killed and their abdomens opened. Except in the duodenal region, the worms could be seen as swellings in the gut. Provided the gut was opened within a few minutes of killing, the muscles caused the wall to reflect and show its contents clearly. The position and appearance of the worms were recorded, the worms were then removed and the glycogen was estimated as previously described.

RESULTS

The experiments, the results of which are shown in Table 1, Fig. 2., were carried out over a period of nine months. Whenever possible a 24, 48 and 72 hour estimation was done at one time, and then another set at a later date, in order to reduce possible errors due to the use of different batches of pigeons at different seasons. The fact that the standard deviations are for the most part low, suggests that the gut environment was fairly constant through the experiments.

It will be seen that the glycogen reserves decrease rapidly at first and then tend to level off. The figures are treated statistically, using the significance formula 13A, given by Chambers (1943), for use with small numbers of samples.

Statistically the Initial/24 hr. figures show a highly significant decrease, but between 24/48 hr. this rate of decrease in reserves has become just not significant, and between 48/72 hr. there is no significance at all. Further analysis shows that the 24/72 hr. figures are significantly different. From this data it may reasonably be assumed that the graph indicates a rapid decrease in glycogen content during the first 24 hours, which is continued for part of the 2nd. day, but that somewhere between 24 and 48 hours, there is a point of inflection, after which statistically insignificant fluctuations are occurring.

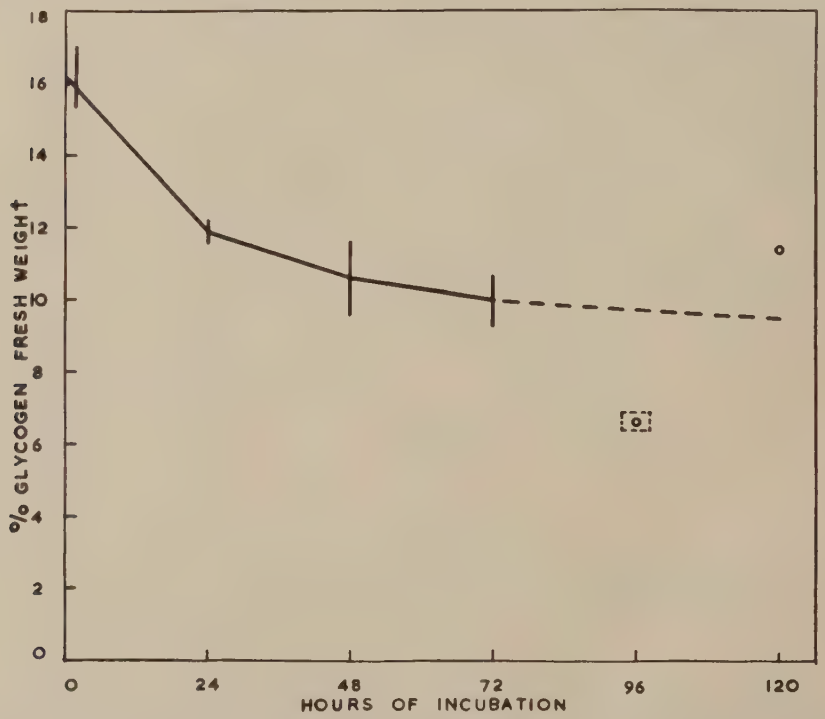

FIG. 2. Changes in glycogen content during maturation of *Schistocephalus* plerocercoids in the pigeon gut. Open circles—single determinations, vertical lines—standard deviations. (See also Table 1).

The 96 hr. figure is ignored on the grounds that the average weight of individual worms in the sample was considerably less than 100 mg. (vide supra). It is of little value to draw conclusions from a single estimation, nevertheless the 120 hr. figure certainly suggests that at least no great fall takes place after 48 hours of incubation.

c. Distribution of worms in the gut

Table 2 shows the total number of worms found in the quarters of the intestine at different times. Absolute distances down the gut are not comparable as intestines of different birds varied in length from 83 cms. to 125 cms.

Although the numbers are small, it appears from inspection that a) the worms are not evenly distributed about the gut, and b) they tend to aggregate irrespective of time in the second quarter. Chi squared test for homogeneity of distribution in the gut shows it to be highly improbable, less than 0.01% P.

The question next arises as to whether the fact that so many worms were found in the second quarter is attributable to chance in sampling. The analysis of variance shows this also to be highly improbable. It would appear therefore that some factor was operating to group the worms into their present distribution.

It may be questioned, since the worms were fed in at one end of the gut, whether this grouping was not purely due to the worms having only reached the second quarter of the gut by the end of the third day. The objection to this is that the frequency peak of occurrence shows no correlation with time.

DISCUSSION

It would seem probable that the initial amount of glycogen is fairly constant, in the region of 16% fresh weight. It is of interest to note that increase in glycogen content in the *Diphyllobothriidae* species appears, from present data, to be paralleled by an increase in plerocercoid development. Thus plerocercoids of *Diphyllobothrium*

TABLE 2.—*Distribution of worms in the pigeon intestine after different periods of incubation.*

Position in intestine	Hours of incubation			Total no. of worms
	24	48	72	
1st. Quarter	1	8	4	13
2nd. Quarter	8	13	13	34
3rd. Quarter	2	6	1	9
4th. Quarter	1	2	0	3

spp. only reach a low level of development in the fish—glycogen content 17.9% Dry Wt. (Markov, 1939). *Ligula intestinalis* reaches a much higher stage of development, genital anlagen being formed (Smyth, 1947),—glycogen 33.7% D. Wt. Finally in *Schistocephalus* the main genital organs are clearly demarcated—glycogen 51.0% D. Wt. Whether there is here a correlation is as yet unknown.

During the first 24 hours of incubation in the host the reserves are greatly reduced. Whether this fall in amount of reserves is due to greatly increased mobility resultant from a 20 degrees rise in temperature—body temperature of pigeons is 41° C. (Simpson and Galbraith, 1905)—or to maturation cannot be determined from these data. After about the 40th hour the fall ceases to be significant. This may be due to the completion of maturation, which is known (Smyth, 1946) to have occurred by then, or to the fact that the worm by this time has arrived in the region of the intestine in which it can absorb nutriment, or to the setting up of a new internal equilibrium level, relative to the new physical conditions, temperature, oxygen, etc.

From the present *in vivo* experiments it is uncertain whether the graph showing depletion of reserves is really measuring a single variable—consumption—or whether at some point nutritional absorption commences, with the result that thereafter the glycogen reserve level will reflect the interaction of consumption and absorption. This reduction in the rate of consumption of the glycogen reserves after 48 hours, maturation, would support Ortner-Schönbach's (1913) hypothesis that the primary function of glycogen was for developing genitalia.

Since all estimations were based on fresh weight, one major qualification is necessary. If the plerocercoids during incubation in the gut absorbed water, then it follows that an apparent decrease in glycogen content would have occurred. As yet lack of worms has prevented this important point being determined directly, but from *in vitro* experiments it would seem improbable that any great change in Fresh/Dry weight ratio does occur, during maturation.

The concentration of worms in the second quarter of the intestine is suggestive of a distribution correlated with that of the simplest products of digestion, monosaccharides, amino acids, etc.

SUMMARY

1. *Schistocephalus* plerocercoids of over 100 mg. mean fresh weight have been analysed. Their composition was—Water 68.22%, Glycogen 16.2%, Protein 11.4%, Ash 1.85%, Undertermined 2.33%.

2. No seasonal variation was shown by the plerocercoids' glycogen reserves.

3. The glycogen content was measured after various periods of incubation in the pigeon's gut. Statistically significant decrease occurs at first, ceasing after 48 hours.

4. The high distribution of worms in the second quarter of the intestine is statistically significant.

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RETROFECTION IN OXYURIASIS. CONCLUSIONS

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In a former communication (Schüffner and Swellengrebel, 1949) we dealt with successful peranal infections with larvae of *Enterobius vermicularis,* as well as with the natural way of retrofection, the source of the chronic form of oxyuriasis in adults, which we termed "type R." Continued observations have now permitted us to extend our knowledge.

Two of our experiments (see figure 1) were still in progress as of December 21, 1949.* In the case of the new volunteer, J. B., the primary attack confirms the prolonged prepatency of 76 days, which was so unexpected in the case of Dr. B. As an exception, however, it shows that the lifetime of the females can extend to 101 days, eight days more than our former record (93 days).

The second part of the experiment on J. B., now in its twentieth month, does not provide any new data. It closely follows the described type R. Nevertheless, it is very surprising that our three cases of peranal infection produced long chains of recidives, whereas our seven peroral cases closed with an acute attack.

The course of the experiment of the senior investigator (Dr. Sch.), which conformed to pure type R in the first year, altered its character during the second year (shown in the accompanying chart, fig. 1): recidives followed one another more rapidly and all rhythm vanished. Finally one worm appeared daily, and so, from February 1949 onwards, the course of the infection changed completely from type R to type D (characterized as "digital") during March and April. We therefore conclude, as we hitherto could only guess, *that pure retrofection also can lead to a heavy worm load.* Without knowing the antecedents, there is no evidence that it was not the finger which carried the infection in this instance. For a proper interpretation of chronic oxyuriasis in adults due consideration must be given to this particular aspect.

This new course of events in Doctor Sch., with the constant increase in direct evidence, namely the appearance of females on the anus, rendered it unlikely that a decline was impending but rather that the marked increase of worm-load could be expected, a condition which was indeed realized in March. Therefore, the time had arrived to undertake the crucial experiment, *i.e.,* an attempt to cut short a further increase of worm-infection by an attack directed at the anus. This took place on February 21, 1949 at which time Doctor Sch. began a systematic "anal-toilette" for a period of 101 days.

We must remember that eggs on extrusion enclose a tadpole-like embryo and that the eggs require a six-hour exposure to the air, in order to provide infective-stage larvae. Although the majority of eggs will be able to develop only after oral ingestion, it appears probable that some of the larvae are capable of emerging in the

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* Professor Schüffner died three days later. He had already sent the manuscript to Dr. E. C. Faust, who has edited it and redrawn the figure. A biographical sketch prepared by Professor Swellengrebel appears elsewhere in this number of the Journal.

moist surroundings of the anus and of entering the bowel by the anal route. This process, retrofection, then initiates the "recidive." To prevent it, all that is required is to remove the eggs at regular intervals before hatching can take place. This can be attained by washing the anus every six hours, *i.e.*, four times daily. Such a theoretical consideration was difficult to carry into effect in the case of Doctor Sch., so the efforts had to be limited to three washings daily, e.g., morning, afternoon and evening.

The details are provided in the chart (fig. 1), running for 29 months. Some 60 days elapsed after initiation of the toilette before the result of this mechanical process became apparent. The recidives, so frequent in March and April, became greatly reduced in number—they dropped to one every four days, then from July 29th onwards on an average to one every 8 days. The essential rhythm is that of a monocycle. Nevertheless, the toilette had its clear results: the experiment had run smoothly, though a residual infection persisted. But this did not in any way vitiate the result which had been expected. We venture to state that in principle the effect of the washings is not subject to question but that in practice three washings are not sufficient to produce eradication of the worms. The fully developed larvae are able to leave the shell from the beginning of the seventh hour, the time allowed them in order to keep up the infection, even when it is reduced to a minimum. Against this only a toilette with shorter intervals would be effective.

We hypothesized that a certain portion of the hatched larvae possess the ability to enter the anus and that, to a certain extent, this peculiarity is inherent. This was rendered more probable by the notable increment of the infection in the case of Doctor Sch. It seems as if a natural selection had been at work, under the influence of which a strain, capable of retrofection, was reared.

In conclusion, there is still one aspect to be considered, to which our attention has been drawn on several occasions. It concerns the objection that all manipulations of the anus, especially washing after evacuation, render it possible for the fingers to be contaminated with eggs in adults as it is in children, and that the increase of the worm-load in the second year in the case of Doctor Sch. could be more easily explained as a finger infection. If this idea were correct, one might have expected that the washings would result in a marked increase. For, instead of cleaning once a day after stool, now thrice a day the fingers came in contact with the anal region, with the unavoidable risk of finger-contamination. The very opposite resulted, viz., a marked decrease took place. From this it may be inferred that in such cases neither fingers nor dust eggs are responsible, but that retrofection is the rule. Hence, it may be concluded that ordinary cleanliness, such as we may expect in adults, but not in children, can check the introduction of eggs into the mouth.

SUMMARY

1. A third experiment, J. B., infected peranally with larvae of *Enterobius vermicularis,* is described. It has been in progress for 20 months as a pure retrofection, type R.

2. In the case of Doctor Sch. the overall picture during the second year altered completely, and type R was converted into type D. The anal toilette every eight hours, which was then instituted, resulted in a marked decrease of recidives. Probably with a six-hour toilette it would have been possible to bring the infection

promptly to an end. Experience allows one to assume that clean adults do not owe their chronic oxyuriasis to finger or dust eggs, but entirely to retrofection. Nothing is known with regard to children and their relation to retrofection.

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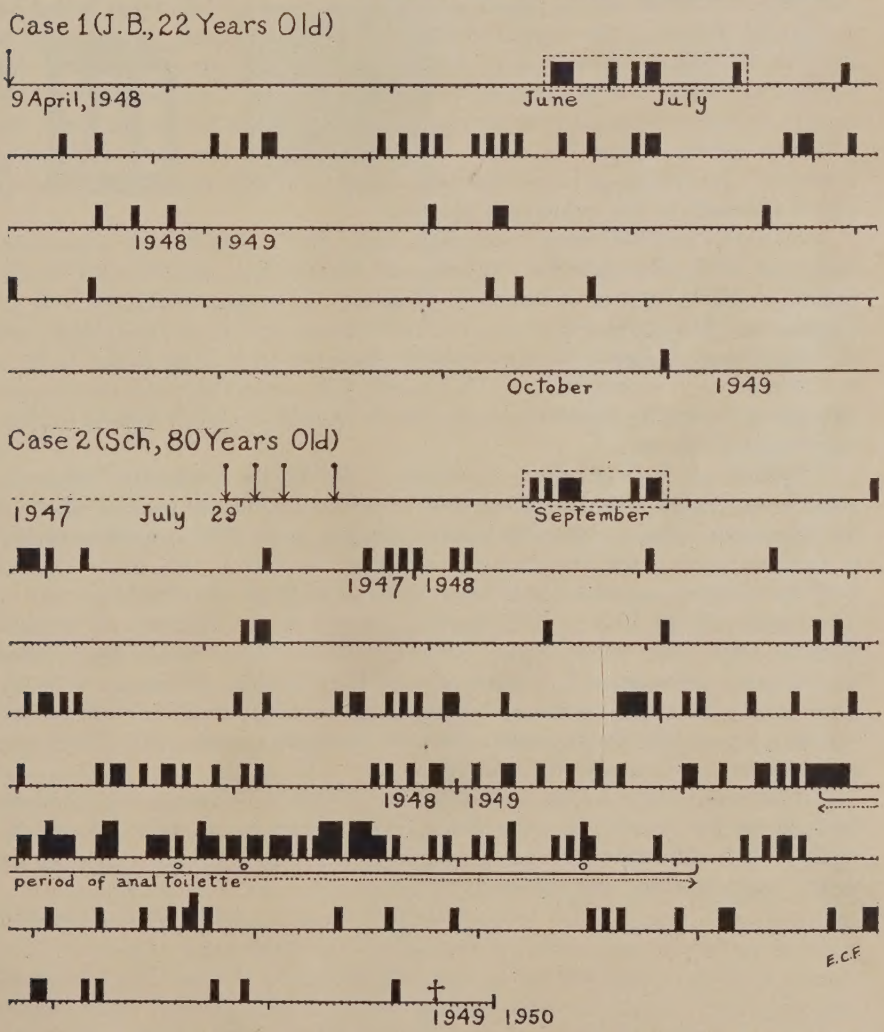

FIG. 1. Day-by-day charting of Case 1 (J. B.) and Case 2 (Sch.). The arrows indicate days when intra-anal inoculation with hatched larvae of *E. vermicularis* took place. The period in each case history blocked in with broken lines covers the primary patency. Each short solid block of one day's span represents the known peri-anal excursion of one female; in Case 2 the taller blocks represent 2 females per day. The 3 small circles during the period of anal toilette in Case 2 indicate failure to perform the evening anal toilette on those days; †, termination of the experiment by death of Dr. Schüffner.

WILHELM AUGUST PAUL SCHÜFFNER

January 2, 1867–December 24, 1949

Schüffner does not rank with those who by their basic research laid the foundation of tropical medicine. But he was the first to shatter the paralyzing belief in the "murderous tropical climate." By controlling infectious diseases and beri-beri, which made mortality soar to disastrous heights, and not by any change in the hot and humid climate of the tropical lowlands of Eastern Sumatra, he managed to improve the health of thousands of field laborers, on the tobacco estates of that country, to a degree equalling that in Northwestern Europe. Today such an achievement is of common occurrence; in the years between 1897 and 1902 it was not. The advantage gained in these five years—with the unstinted support of the Board of Directors—was confirmed in the next four decades. It took no less than the war and its aftermath to ruin Schüffner's life-work.

Schüffner's scientific activity covered a wider field. Structures in malaria-infected red blood cells (Schüffner stippling and Maurer dots, both of which he described in 1899); the identification of Noguchi's *Leptospira icteroides* with *L. icterohaemorrhagiae;* the discovery of the rodent reservoir of *L. grippo-typhosa,* and of "retrofection" in *Enterobius vermicularis,*—these are some of the salient features in a long series of investigations. They would have sufficed to make him famous throughout the world. But his achievements in the field of applied tropical hygiene overshadow all others.

Schüffner was M.D. of Leipzig (Germany). In 1912 the Amsterdam University made him an honorary M.D. From 1897 he served as principal medical officer of the Semembah Tobacco Estates of Eastern Sumatra, from 1916 as medical adviser to the Government of the Netherlands East Indies. In 1922 he came to the Netherlands where he was appointed to the chair of Tropical Hygiene at the University of Amsterdam. From 1924 to 1937 he was director of the Institute of Tropical Hygiene, Department of the Royal Institute for the Indies, at Amsterdam. After his retirement at the age of 70, a laboratory, specially provided and equipped for the purpose by private subscription, and incorporated in the institute of which he had been director, enabled him to continue his work for another seven years. Then war and post-war conditions ended his career.

Schüffner was born a German and he died a German. True to the "Fatherland" to the last, he was painfully aware of the crimes it was guilty of, and he admitted them in that phrase of proud resignation with which he used to counter his critics, "right or wrong, my country."

N. H. SWELLENGREBEL